# Assessment of Body Composition and Nutritional Status in Individuals with Obesity Before and in the Long-Term After Bariatric Surgery

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# DEDICATION

This dissertation is dedicated to my husband Caleb Johnson.
I could not have done this without you.

ABSTRACT

One of the most successful treatments for obesity is bariatric surgery. The Roux-en-Y gastric bypass (RYGB), in particular has been the most commonly performed bariatric surgery over the past decade. It is not well known how time will influence the broader inflammatory-related health outcomes of bariatric surgery, which may depend on complex inter-relationships between nutritional status and body fat mass (FM). This dissertation focuses on the long-term changes in nutritional status and body composition after RYGB with a particular focus on vitamins and minerals that have potential inflammatory action and the development of improved methods to assess changes in body composition in obese individuals. In Chapter 3 we report the results of a pilot study to assess long-term changes in body composition and nutritional and inflammatory status after RYGB. From the assessment of 5 women who were monitored over an 8.5-year period after RYGB we found that improvements in vitamin D status and potential improvements in inflammatory status can occur over time. However, continued loss of lean soft tissue (LST) occur on the background of weight regain between 1-year and 8.5-years post-RYGB. Losses of LST were correlated with decreased handgrip strength. In Chapter 4 we report the results of a validation study to compare the results of a new application of bioimpedance spectroscopy (BIS) based on multicomponent physiologic models with existing body composition data from dual energy X-ray absorptiometry (DXA) in a large NHANES dataset with 5470 observations and in a longitudinal dataset of 25 women for the first-year after RYGB. We found that the BIS method was in

relatively good agreement with DXA for the assessment of FM and lean tissue, and that the BIS method was equally as good as DXA for assessing changes in FM in particular after RYGB, over the period from 6-months to 1-year. In the coming years, bariatric surgery is sure to remain a popular treatment for obesity and it is clear that we need better methods to assess changes in body composition in a more comprehensive way, in order to better understand the ramifications of these changes in light of long-term nutritionally relevant health outcomes, including inflammation. This dissertation could serve to inform future studies that should aim to tease apart the factors contributing to long-term FM gain, but more importantly loss of LST and muscle strength, to establish evidence based guidelines.

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# LIST OF ABBREVIATIONS

AACE – American Association of Clinical Endocrinologists

ADP – Air displacement plethysmography

ANOVA – Analysis of variance

ASMBS – American Society for Metabolic and Bariatric Surgery

B-A – Bland-Altman analysis

B-A PE – Bland-Altman percent error

BIS – Bioimpedance spectroscopy

BMC – Bone mineral content

BMI – Body mass index

BPD/DS – Biliopancreatic diversion with or without douodenal switch

CCC – Concordance correlation coefficient

CDC – Centers for Disease Control and Prevention

$C_M$ – Cell membrane capacitance

cm – centimeter

COX-2 – Prostaglandin endoperoxide synthase-2

CPBA – Competitive protein binding assay

CRP – C-reactive protein

CT - Computed tomography

CTSI – Clinical and Translational Science Institute

CV – Coefficient of variation

d – day(s)

DXA – Dual energy X-ray absorptiometry

ECW – Extracellular water

ELISA – Enzyme-linked imunosorbent assay

eNOS - Endothelial nitric oxide synthase

ExF – Excess fluid

FFM – Fat free mass

FM – Fat mass

g – gram(s)

GCRC – General Clinical Research Center

HAI – Hospital acquired infection(s)

HPLC – High performance liquid chromatography

ICW – Intracellular body water

IFN-γ – Interferon gamma

IL – Interleukin

IRB – Institutional Review Board

ITGAM – Integrin alpha M

IU – International unit

IV – Intravenous

keV – kiloelectron volt

kg - kilogram

LAGB – Laparoscopic adjustable gastric banding

LABS – Longitudinal assessment of bariatric surgery study

LC-MS/MS – Liquid chromatography tandem mass spectroscopy

LDL – Low density lipoprotein

LST – Lean soft tissue

M1/M2 – Type 1 and type 2 macrophage

MCP-1 – Monocyte chemoattractant protein-1

MCRU – Masonic Clinical Research Unit

MF-BIA – Multi-frequency bioelectrical impedance analysis

ml – milliliter(s)

MRI – Magnetic resonance imaging

mRNA – Messenger ribonucleic acid

n – number of subjects

NaCl – Sodium chloride

NH_AT – Normally-hydrated adipose tissue

NH_LT – Normally-hydrated lean tissue

ng – nanograms

NHANES – National health and nutrition examination survey

NK – Natural killer

PMN – Polymorphonuclear neutrophils

R – Resistance

*r* – Pearson's correlation

ra – Receptor agonist

REE – resting energy expenditure

RMSE – Root mean square error

RQ – Respiratory quotient

RT-PCR – Real time polymerase chain reaction

RYGB – Roux-en-Y gastric bypass

SD – Standard deviation

SF-BIA – Single frequency bioelectrical impedance analysis

SOS – Swedish obesity study

T2DM – Type two diabetes mellitus

TBW – Total body water

Th1/Th2 – Type 1 and type 2 T-helper cells

TNF-α – Tumor necrosis factor alpha

TOS – The obesity society

$VCO_2$ – Volume of carbon dioxide

$VO_2$ – Volume of oxygen

vol – volume

VSG – Vertical sleeve gastrectomy

wt – weight

X – reactance

25(OH)D – 25-hydroxyvitamin D

μl – microliter

US – United States

# CHAPTER 1 : INTRODUCTION

Bariatric surgery is one of the most successful treatments for obesity. The long-term ramifications of bariatric surgery for broader nutrition-related health outcomes are not well studied, in part due to logistical difficulties in following individuals for years after surgery. The impact on body composition and nutrition, and the potential implications of the complex interactions between nutrition and inflammation may be important. Although other procedures have recently gained in popularity, the Roux-en-Y gastric bypass (RYGB) surgery continues to be utilized at a high frequency, given its documented long-term success for inducing both weight loss and diabetes remission. The bypass of the small intestine during this procedure can and does result in variable rates of nutrient deficiencies. For example, deficiency of vitamin D is documented in the literature and may have implications for inflammatory status in the long-term after surgery.

This project is unique in that it reports on body composition and nutritional status changes 8.5-years post-RYGB. From other studies we know that by 1-year the primary change in body composition is a substantial loss of fat mass (FM), accompanied by smaller losses in lean tissue. Weight regain in the long term after RYGB is documented in the literature; however, we do not know which tissue compartments are responsible for that weight gain and it is not known if the lean tissue losses that occur in the first year after bariatric surgery continue in the long-term or stabilize. It is not clear how lean tissue changes in the long-term affect functional status and strength. Muscle loss is one of the

major criteria in the diagnosis of malnutrition, thus it is of paramount importance to monitor these changes as people age. Accurate bedside methods for the assessment of lean tissue loss are lacking. The ability to measure lean tissue would be advantageous for evaluating the response to nutritional interventions in clinical settings, and to weight management interventions (including physical activity, nutrition, and surgical) in individuals with obesity. Knowledge of the timing and magnitude of lean tissue changes after bariatric surgery would be also be quite valuable for monitoring health outcomes.

This dissertation focuses on the long-term changes in nutritional status with a particular emphasis on vitamins and minerals with potential inflammatory action, body composition and muscle strength changes that occur after bariatric surgery, and the development of improved methods to potentially assess changes in body composition in individuals with obesity. Chapter 2 is a literature review that will present background information to frame the two research chapters. This chapter focuses on the types of bariatric surgeries; how body composition is measured; how body composition changes after bariatric surgery in the short- and long-term; and how nutritional status also changes in the long-term after surgery with possible inflammatory complications. Included in this chapter is an in-depth published review of vitamin D status changes after bariatric surgery and the potential implications of these changes. Chapter 3 presents a long-term assessment of body composition changes and select nutrient status after RYGB with a focus on vitamin D changes and inflammation. Chapter 4 is a validation study comparing new models for the generation of body composition components from bioimpedance

spectroscopy (BIS) data to dual energy X-ray absorptiometry (DXA) data in a large NHANES dataset and in a longitudinal dataset of weight loss after RYGB. Chapter 5 is a discussion of the overall conclusions that can be drawn from these studies and the potential future directions for research.

# CHAPTER 2: LITERATURE REVIEW*

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## 2.1: Bariatric Surgical Procedures

Historically, bariatric surgeries have been described as either malabsorptive or restrictive. As more is learned about the mechanisms driving the success of bariatric surgeries, these classifications may not hold. For many years, the most common bariatric surgeries have been the Roux-en-Y gastric bypass (RYGB) and the biliopancreatic diversion (BPD) with and without douodenal switch (DS). The RYGB and BPD/DS are often termed *restrictive* and *malabsorptive*. However these classifications are limiting and do not accurately address all the numerous mechanisms thought to be involved in the metabolic improvements seen after bariatric surgery. The most common types of so called *restrictive* surgeries are vertical sleeve gastrectomy (VSG) and laparoscopic adjustable gastric banding (LAGB).[1] Again, the historic classification system may be too simplistic, particularly when discussing the VSG because newer research suggests that mechanistically there is more involved in the success of this surgery than simply caloric restriction and that rapid gut transit and gastric emptying have been shown after VSG.[2,3] Throughout the years there have been numerous other iterations of bariatric surgeries. For the sake of simplicity, here the discussion will focus on the four most commonly performed procedures.

### *2.1.1: Roux-en-Y Gastric Bypass*

During the RYGB a small gastric pouch, approximately 15 ml, is created and connected to the jejunum via what is commonly termed the Roux-limb. Gastric and pancreatic secretions as well as bile are secreted into the pancreatobiliary limb, which is

anastomosed with the jejunum. The major mechanism for weight loss following RYGB is thought to be the reduction in stomach size, which leads to smaller meal size and fewer calories consumed. Additionally, the length of the Roux limb is thought to be proportional to the degree of malabsorption following surgery. Finally, the physiologic changes following surgery appear to alter gut hormones,[4] increasing those that promote satiety and reducing those that suppress hunger.[5] In addition, RYGB has shown remarkable success for inducing remission of type 2 diabetes mellitus (T2DM).[6]

### *2.1.2: Vertical Sleeve Gastrectomy*

During the VSG procedure the stomach is resected longitudinally, removing 80% to form a tubular pouch. The procedure preserves the antrum and the pylorus. The newly created gastric pouch has an approximate volume of <100ml. The sleeve has been shown to be as effective as the RYGB in terms of weight loss and T2DM remission rates.[7,8] As with RYGB, the VSG has an impact on gut hormones, which impact metabolic signaling pathways.[7]

### *2.1.3: Biliopancreatic Diversion With and Without Douodenal Switch*

With the BPD and the DS procedures, 70% of the stomach is resected much like the VSG to create a tubular pouch. Much of the small intestine is bypassed. BPD can be performed with and without DS where the proximal duodenum is anastomosed to the distal small intestine to create a short common channel. This procedure allows for preservation of the pylorus and reduces risk of dumping syndrome. The bypassing of the small intestine limits absorptive area where the food can mix with bile secretions and

pancreatic enzymes, which results in increased malabsorption. Similar to the VSG and RYGB, the BPD/DS is successful in resolving T2DM and is often considered the most effective surgery for treating T2DM.[6] The BPD/DS also affects gut hormones in a way that appears to influence hunger and satiety.[6]

### *2.1.4: Laparoscopic Adjustable Gastric Banding*

LAGB is a reversible procedure and is often the first choice for patients and surgeons.[9] During the LAGB procedure, an inflatable band is secured around the top of the stomach allowing for variable restriction of the movement of food from the small gastric pouch into the remainder of the stomach and the duodenum. Weight loss is slower after LAGB than other surgeries as the band is slowly inflated to restrict the stomach in the weeks after surgery. The mechanism for LAGB success is unclear but is thought to be related to increased satiety.[10,11]

### *2.1.5: Prevalence of Bariatric Procedures*

Until very recently, the most common procedure performed in the United States and worldwide was the RYGB, although the VSG is quickly surpassing the RYGB in popularity due to its nearly equal or better efficacy for weight management[12] and diabetes resolution. VSG is further favored due to its lower complication rate.[7] In 2013, the most recent year for which estimated numbers are available from the American Society for Metabolic and Bariatric Surgery (ASMBS), there were approximately 179,000 bariatric surgeries performed in the United States. Of these, 34.2% were RYGB, and 42.1% were VSG. 14% were LAGB and only 1% were BPD/DS. Approximately 6% of bariatric

surgeries are revisions and a small amount (2.7%) are classified as other.[1] The prevalence of revision has also been reported and has been recently reviewed by O'Brien.[9] After 10-years, the reported revision rates range from 8-38% for RYGB, 8-60% for LAGB, and 10-40% for gastroplasty.[9] As with other metrics, long-term data for revision rates is not available for VSG.

## 2.2: Commonly Used Body Composition Assessment Methods

One of the challenges facing clinicians with the increasing prevalence of obesity in the US and worldwide is the need to evaluate changes in body composition in individuals with extreme obesity. A large majority of the reference methods for the evaluation of body composition have been developed using normal weight, healthy individuals. Thus, there is a need to validate, refine, and improve these methods so they can be utilized in the obese population.

### *2.2.1: Reference Methods for Assessing Body Composition: DXA, Body Density, Dilution and 4-Component Models*

As has been well described by Heymsfield and Wang, the human body can be thought about in terms of the five-level model including the *atomic* or *elemental* level (oxygen, hydrogen, carbon, nitrogen and other elements), the *molecular* level (lipid, water, proteins, glycogen, and minerals), the *cellular* level (cells, extracellular fluid, extracellular solids), and the *tissue-system* level (adipose, skeletal muscle, visceral organs, skeleton) and finally, the *whole body* level.[13] Many methods have been developed for the assessment of body composition. Detailed reviews of these methods, their history, and use are widely available.[13,14] Traditional body composition models are based on 2-compartments where body weight is divided into two fractions, FM and FFM. These are often referred to as 2-component models and they assume that the density of FM and FFM are stable for all adults.[15] These models may be too simplistic and may fail to adjust

adequately for ethnicity, aging, gender, and excess adiposity, leading to the development of multi component models based on multiple reference techniques.[16,17]

Body composition methods range in complexity from field anthropometric techniques like skin fold thickness and waist-to-hip ratio, to technologically advanced imaging techniques like computed tomography (CT) and magnetic resonance imaging (MRI).[18] The most commonly used reference methods to assess body composition fall in between these methods in terms of cost, reliability, and accessibility to researchers: these include the total body water (TBW) and extracellular water (ECW) assessment by deuterium and bromide dilution, respectively (i.e. multiple dilution), body density by underwater weighing or air displacement plethysmography (ADP), DXA, and multi-component models based on these techniques.[14,18] Here we will focus on the following reference methods of body composition assessment: multiple dilution, body density measured by ADP, DXA and a 4-component model based on these methods.

DXA provides information about body composition divided into 3-compartments where body weight = FM + bone mineral content (BMC) + lean soft tissue (LST).[17] LST is composed of water, proteins, glycogen and minerals, and FFM is LST with the addition of BMC.[17] DXA typically produces photons at 40 and 70 keV, which pass through tissues at rates depending on their elemental composition.[14] DXA output allows for visualization and analysis of the separate tissue types.[14,19] While DXA has its own limitations and sets of assumptions, it is well regarded as a method to accurately assess BMC, FM, FFM, and

LST. Height, weight and width limitations have historically restricted the use of DXA to those individuals who are mobile and who meet the body physical size specifications required to use the machine. However, newer machines (e.g. iDXA, GE Healthcare) are capable of measuring larger individuals.

Dilution methods measure the ratio of a dose of a tracer to the concentration in the body compartment after a defined follow-up time. A known concentration of tracer is compared with the difference between levels of the tracer (in urine, saliva or blood) before (background) and after dosing. Dilution methods rely on the assumptions that that the tracer is distributed only in the body compartment of interest, that it is equally distributed in this compartment, that it is not metabolized during the monitoring period, and that the time to equilibrium is achieved quickly and reached by the time of measurement.[14]

Dilution is one of the more common methods to assess TBW. TBW is an important measurement for body composition as FFM has been shown to be hydrated at 73% in healthy adults.[20,21] TBW can be measured with a variety of tracers including tritium, deuterium or oxygen-18. Deuterium is ideally given as an oral diluted dose of 99.9 atom% deuterium oxide in tap water. Typical equilibration times are 3-hours after oral deuterium dosing for TBW; equilibration time may be shorter with IV dosing and are extended by 1-hour in individuals with obesity.[22] ECW is typically measured in the same fashion using non-radioactive bromide (Br) delivered as a 3% sodium bromide oral dose

solution in tap water. Typically Br dilution is assessed in plasma or serum 3-4 hours after dosing, with 1-hour extension in individuals with obesity of known fluid overload. It has been documented that Br equilibrium may not be reached until 8.5-12 hours after dosing, although longer equilibration periods are rarely used.[23,24] Inadequate equilibration time could lead to over-estimation of the ECW compartment. Intracellular water (ICW) can be determined using radioactive potassium ($^{42}K$), although this method is not frequently used. Alternatively ICW can be calculated from deuterium and Br dosing as the difference between TBW and ECW.

Hydrodensitometry (under-water weighing) has long been considered the gold-standard for assessment of body density. Hydrodensitometry is technically challenging and difficult for study subjects to accept because it involves complete submersion in water with maximal expiration, ideally followed by measurement of residual lung volume. In recent years, the availability of ADP has provided an alternative to hydrodensitometry that is more acceptable to study participants. ADP has been shown to have good agreement with hydrodensitometry, thus it has been accepted as a reference method for density.[25–27] ADP devices use the relationship between pressure and volume, at a fixed temperature, to solve for the volume of air displaced when an individual sits in a chamber. ADP has been reviewed by numerous groups.[13,14] In brief, FM and FFM are calculated from the volume and density estimates using established prediction equations (e.g. Siri[15]). These devices are also capable of measuring thoracic gas volume, which is important for the correction of body volume.

Multi-component models are often considered superior to single reference methods because by measuring several compartments there is less need for reliance on assumptions, e.g., the FFM hydration constant. For example, a 4-component model, aim to account for aqueous, protein, mineral, and fat fractions by using different reference methods to measure each.[17] One 4-component model commonly utilized is based on TBW by deuterium dilution, body density by ADP, BMC by DXA, and body weight.[28] As with any method, there are of course issues with the multi-component models. With more independent measurements there exists a greater risk of measurement error; however, overall error of the model is thought to decline with the incorporation of more measurements.[16]

*2.2.2: Field Technique: Bioimpedance Spectroscopy*

Limitations of existing body composition reference methods is that they are costly, difficult to transport, and require specialized maintenance and training for optimal performance.[13] Bioimpedance analysis techniques such as bioimpedance spectroscopy (BIS) are commonly referred to as field techniques because they require validation against reference methods and are typically portable, non-invasive, lower in cost, and applicable for bedside use. We have recently reviewed these techniques and their potential for application at the bedside.[29] Most of literature focuses on single- and multi-frequency bioelectrical impedance analysis (SF-BIA and MF-BIA) approaches that rely on population specific regression derived prediction equations to estimate body compartments from impedance data. Although these techniques may produce reasonably

accurate and precise estimates in healthy individuals, the underlying assumptions (e.g. one or a few concise frequencies are sufficient to adequately capture body water, and relationships between water compartments, bone and protein are static).

Unlike SF-BIA and MF-BIA, BIS involves the application of biophysical modeling algorithms to bioimpedance data measured across a spectrum of frequencies (e.g. from ~5 to 1200 kHz, using data from 50 or more frequencies) in order to generate body composition data. Simply put, at low frequencies (e.g. approaching theoretical 0 frequency), the current produced by a BIS device is conducted only by ECW, due to the capacitance effect of cell membranes and tissue interfaces. At relatively high frequencies (e.g. approaching theoretical infinity frequency), BIS devices measure ICW as the capacitive property of cells is lost at higher frequencies, allowing the current to pass through cell membranes and tissues and thus quantifying both ICW and ECW (i.e. TBW).

The bioimpedance data are typically fit to the Cole model[30] using nonlinear least squares curve fitting; the semicircle formed by plotting resistance (R) and reactance (X) values is extrapolated to reach the x-axis in order to determine R at zero ($R_0$, also called $R_E$) and infinity ($R_\infty$). From these, $R_I$ can be calculated, representing the resistance related to the ICW. Cell membrane capacitance ($C_M$) is also calculated in the model. Cole model terms can be applied to the Xitron-Hanai-based[31,32] mixture equations to determine ECW and ICW; these equations are based on the conductivity of suspensions[33] and consequently can be used in conjunction with the presumed conductivity and resistivity

of the different body compartments (i.e. ECW and ICW). These equations incorporate several constants including intra- and extra-cellular apparent resistivity, shape factor, and body density. The apparent resistivity constants for the ICW and ECW compartments that are typically applied to the mixture equations require the assumption that the resistivity of all tissue types is constant, however, it has been shown that these constants may vary with adiposity, and the errors in ICW and related lean tissue compartments increase with increasing adiposity.[34–36]

### *2.2.3: Limitations for Assessing Body Composition in Obese Individuals*

Until recently DXA was limited to use in individuals who were below weight and size thresholds required to meet device specifications – typically below 300 lbs and less than 60 cm wide.[19] The newer DXA devices such as the iDXA (GE, Lunar Medical Systems, Madison, WI) can accommodate larger individuals up to 450 lbs and 66 cm wide, maximal torso thickness is constrained only by the height of the scanning arm which is set at 46 inches.[19] FM thickness and distribution may also impact the validity of DXA in assessing body composition, including bone mass.[37] Other methods such as multiple dilution using deuterium depend on a set of standard assumptions for the hydration of FFM (0.738[20]) and body density (1.1 g/ml[15]) that may be violated in extremely obese individuals who tend to have elevated levels of ECW,[38] which persists even after weight loss as previously discussed.[39,40] Increased adiposity also introduces error due to violation of underlying assumptions in bioimpedance techniques. For example, in individuals with obesity, hydration of FFM is higher than 73%, body

proportions are skewed compared to normal weight, and resistivity constants for intra and extracellular compartments applied to BIS equations are likely affected by increasing adiposity and could be more variable across tissues.

## 2.3: Changes in Body Composition Following Bariatric Surgery

The most recent data available from the Centers for Disease Control and Prevention (CDC) for 2011-2012 showed that 35% of adults 20 years and older in the US were classified as obese (body mass index [BMI] $\geq$ 30 kg/m$^2$) and 6.4% were extremely obese (Class III obesity, BMI $\geq$ 40 kg/m$^2$).[41] One of the most effective treatments for obesity is bariatric surgery. Bariatric surgery is typically only recommended for individuals with BMI $\geq$ 40 kg/m$^2$ or for those individuals with a BMI $\geq$ 35 kg/m$^2$ with one or more serious comorbidities (e.g. T2DM, hypertension, hyperlipidemia, etc.).[42] Although, it should be noted that there are some professionals who currently support lowering this cut-point to a BMI of 30 kg/m$^2$,based on growing evidence of lower risk of morbidity and mortality with currently applied procedures.[9] This lower risk is thought to be due to the advances in laparoscopic techniques used in modern version of the bariatric surgieries.[9]

In addition to weight loss, one important benefit of bariatric surgery is the improvement of insulin sensitivity, and in many cases, the resolution of T2DM. In a meta-analysis of 621 bariatric surgery studies published between 1990 and 2006, it was shown that the BPD/DS was most effective for both weight loss and diabetes resolution.[6] For all bariatric surgeries assessed in the meta-analysis, 78% resulted in the resolution of diabetes.[6] Furthermore, total weight loss for all bariatric surgeries, for which data were available, was 38.5 kg or 55.9% of excess body weight lost, defined as weight loss divided by ideal body weight or weight at BMI of 25 kg/m$^2$, expressed as a percent.[6]

*2.3.1: Loss of Fat and Lean Tissue After RYGB*

Numerous studies have attempted to quantify the changes in fat mass (FM) after bariatric surgery. Excess FM is associated with low-grade inflammation, T2DM, hypertension, non-alcoholic fatty liver disease, and increased risk for cardiovascular disease. The loss of FM following bariatric surgery is beneficial for the potential resolution of many of these complications and is well documented. However, the FM loss following bariatric surgery is also accompanied by the loss of fat free mass (FFM) (comprised of LST and BMC as measured by dual-energy X-ray absorptiometry [DXA]), which may contribute to potentially significant complications after surgery such as increased all-cause mortality,[43] although this finding is not confirmed in bariatric populations. Loss of FFM also occurs with T2DM and aging,[44] thus the population undergoing bariatric surgery may be at particular risk for lean tissue losses.

There have been a number of reports in the literature using various reference methods to assess changes in body composition in adults after RYGB. FM loss as a percent of weight loss has been reported to be between 73-80% at 6-months to 1-year after RYGB.[39,45–51] FFM loss has been reported to be between 18-25% of weight loss 1-year after RYGB;[39,46–48,51] and LST loss has been reported to be between 12-23% of weight loss 1-year after RYGB.[49,50] Olbers et al reported body composition changes in 29 individuals (22 female, 7 male; mean BMI 42.3 ± 4.5 kg/m$^2$) 1-year after RYGB as measured by DXA and found that total FM decreased by a mean of 26.9 ± 9.4 kg (~73%

of weight loss) and LST decreased by a mean of 4.4 ± 2.6 kg (~12% of weight loss).[50] Carrasco et al also reported body composition changes over 1-year after RYGB measured by DXA in 42 women (BMI 45.0 ± 4.3 kg/m$^2$) and found that FM decreased by 28.6 kg (73% of weight loss) and FFM decreased by 10.7 kg (27% of weight loss).[48] Zalesin et al followed 32 individuals (mean age, 46.7 ± 10.4 years; initial BMI 50.1 ± 8.9) between 3- and 14-months after RYGB and used DXA to assess body composition. They did not report mean changes in LST or FM for the overall dataset, but did report that mean FFM loss was 3.1 kg (13% of weight loss) over the time period.[52] They also reported that only 3/32 patients maintained or gained lean mass as measured by DXA after RYGB.[52] The correlation between rates of FFM and FM lost was 0.37 (P=0.049) and patients losing weight at the fasted rates appeared to have accelerated losses of both lean and FM.

Carey et al followed 17 individuals (12 female, 5 male; BMI 48.5 kg/m$^2$) over 1-year post-RYGB and measured body composition using underwater weighing. They reported that FM loss and reported that between over 1-year FM loss was 38.3 kg (~75% of weight loss) and FFM loss was 12.7 kg (~25% of weight loss). Due to the limitations of underwater weighing, no data on LST is available for this dataset. Das et al reported changes in body composition in a cohort of 20 women (BMI 47.8 ± 8.8 kg/m$^2$) using a 3-component model based on ADP and deuterium dilution after weight stabilization post-RYGB (~14 months). FM decreased by 35.7 kg (80% of weight loss) and FFM decreased by 9.1 kg (20%) at weight stabilization (BMI 30.5 ± 7.0 kg/m$^2$).[39] Carrasco et al assessed changes in body weight using TBW measured by deuterium dilution and the hydration

constants of 0.756 and 0.747 reported by Das et al[39] to calculate FFM and to indirectly calculate FM as the difference between FFM and body weight.[47] In 30 individuals (BMI 44.4 ± 4.8 kg/m$^2$) over a 6-month period after RYGB, FM loss was 26.0 kg (77% of weight loss) and FFM loss was 7.8 kg (23% of weight loss).[47]

In another study similar changes have been reported in a small cohort of 5 adolescents/young adults (all female; BMI 49.8 ± 5.4 kg/m$^2$) between 13-21 years of age.[49] Inge et al measured changes in body composition measured by DXA and found that that by 1-year after RYGB, overall mean FM loss was 36 kg (74% of weight loss) and LST loss was 11.1 kg (23% of weight loss).[49]

A potential long term consequence of bariatric surgery that has not been addressed in the literature is sarcopenic obesity, defined as low muscle mass or function in the presence of obesity. This condition is associated with diminished quality of life and functional status and is compounded by inactivity, low protein intake,[53] and aging.[54] While exact recommendations vary, it has been proposed that a minimum protein intake of 80-90 g/d may be beneficial for preservation of lean mass.[42,55] Unfortunately due to changes in stomach size and satiety, protein intake substantially decreases after surgery,[56] and most individuals do not meet the minimum recommendations even at 1-year post-RYGB.[57] While some studies suggest that protein supplementation could be beneficial for the maintenance of lean mass, consensus has not been reached due to a lack of causative evidence to support this relationship.[42] More research is needed in this area.

Exercise is also likely to be important for the preservation of lean mass after bariatric surgery. Many individuals who undergo bariatric surgery do not get adequate exercise before or after surgery.[58] Exercise is recommended before and after bariatric surgery[42] as it has been associated in this population with increased excess weight loss,[59,60] prevention of strength loss,[61] improved cardiac and pulmonary function, increased resting energy expenditure (REE), and improved glucose tolerance.[62,63] However, while exercise has been regarded as beneficial for post-bariatric individuals, there is not yet solid evidence to suggest that it is linked to prevention of lean tissue losses that typically accompany losses in FM.

### *2.3.2: Altered Fluid Status in Extreme Obesity Persists After Massive Weight Loss*

Altered fluid status in the obese state before and after bariatric surgery has been reported by a number of groups. Das et al measured changes in body composition in 20 women before (BMI 48.7 ± 8.8 kg/m$^2$) and after weight loss (BMI 30.5 ± 7.0 kg/m$^2$) from RYGB and reported that FFM hydration was significantly higher than the standard reference of 0.738 before weight loss (0.756, $P<0.001$) but not after weight loss.[39] Mazariegos et al studied the changes TBW, ECW, and ICW in 25 extremely obese women (BMI 48 ± 7 kg/m$^2$) before and after bariatric surgery (either restrictive or malabsorptive). Compared with normal weight controls, the extremely obese group had elevated ratios of ECW/ICW (0.82 ± 0.17 v. 0.63 ± 0.06, $P<0.05$).[40] Furthermore, this group reported that the elevated ECW/ICW ratio was related to the type of surgery

performed, and was higher (1.09 ± 0.25 v. 0.83 ± 0.14, P<0.01) after weight stabilization at a mean of 22 months after malabsorptive procedures compared to restrictive procedures.[40] Levitt et al also assessed the ECW/ICW ratio in 20 women (BMI 46.7 $kg/m^2$) after RYGB and found that the ratio was higher in the obese individuals at baseline than might be expected for lean individuals (0.82 ± 0.19) and increased 1-year after surgery (1.08 ± 0.3, P=0.002).[45]

### *2.3.3: Weight Regain After Bariatric Surgery*

Long-term data for bariatric surgical success as measured by weight loss maintenance over time indicates that most patients regain at least a portion of their body weight in the long-term after surgery.[64–66] Few well designed, and well-controlled studies have assessed weight loss and health outcomes between 3-15 years post-surgery.[67,68] The older surgeries, such as the RYGB are typically discussed, as are gastric banding procedures such as LAGB, although newer surgeries like the VSG are rarely mentioned, likely due to the lack of long-term data for these individuals. Most studies report weight change and changes in metabolic status (i.e. diabetes remission), but do not look at more complex metrics or body composition as the primary datasets are often from existing medical records or self-report. There is some evidence that weight regain may mitigate the positive health outcome effects of bariatric surgery (particularly of RYGB and LAGB).[67] The underlying causes of weight regain are not well understood and are thought to be multifactorial and related to patient- (e.g. psychiatric, physical inactivity,

metabolic, dietary, etc.) and procedure- (e.g. surgery type, Roux limb length, etc.) specific factors.[68]

The Swedish Obese Subjects study (SOS) is the largest non-randomized study of weight loss outcomes after bariatric surgery (n=4000).[69] They reported 10-year data showing that individuals who underwent RYGB regained ~12% total body weight and those who underwent variable or fixed banding regained ~8% total body weight. For patients following RYGB, this means that by 10-years, individuals had regained ~34% of their maximal weight loss at 1-year.[64] Cooper et al reported weight regain data for a cohort of 300 individuals who underwent RYGB and found that mean weight regain over a mean of ~7 years post-RYGB was 23.4% of maximum weight loss.[66] Roslin et al reported that in 36 individuals post-RYGB mean weight change from lowest body weight after surgery was 8.2 ± 8.6 kg over a mean follow-up period of 3.4 ± 2 years.[69] The Longitudinal Assessment of Bariatric Surgery study (LABS) assessed 3-year outcomes in individuals after either RYGB (n=1738) or LAGB (n=610).[65] Median percent weight loss was 3.15% and 15.9 % of baseline weight for RYGB and LAGB, respectively. Maximal weight loss was observed 1-year post surgery for both RYGB and LAGB, although from 2 to 3 years post-surgery while LAGB weight remained stable, RYGB showed some evidence of weight regain.[65] Others have also reported long-term weight maintenance after LAGB, with one study reporting 47.1% of excess weight loss at 15-years after surgery.[70]

## 2.4: Long-Term Development of Nutritional Deficiencies with Implications for Inflammatory Status Following Bariatric Surgery

Manipulation of the gastrointestinal tract during bariatric surgery can alter the absorption of nutrients that can lead to the development of nutritional deficiency after surgery. Nutritional deficiencies following bariatric surgery have been extensively reviewed in the literature.[71,72] Ideally, supplementation begins early after surgery and continues as part of ongoing post-surgical care.[42] Many individuals require life-long additional supplementation above the levels found in over-the-counter vitamin and mineral supplements.[71] Despite this supplementation nutritional deficiencies can persist after bariatric surgery.[73]

Of particular concern are: vitamin $B_{12}$, thiamin, vitamin C, folate, vitamin A, vitamin D, vitamin K, and trace minerals including iron, selenium, zinc, and copper.[71] After RYGB and VSG, individuals are instructed to consume two chewable adult multivitamins with minerals, 1200-1500 mg of elemental calcium, at least 3000 IU of vitamin D, and vitamin $B_{12}$ as sublingual, subcutaneous or intramuscular preparations (or orally if shown to be adequately absorbed).[42] For LAGB, supplementation is the same, but only one multivitamin is required per day and additional vitamin $B_{12}$ supplementation is not recommended.[42] If nutritional deficiencies are identified during follow-up, individuals are instructed to consume additional supplementation tailored to their specific needs.

Of these vitamins and minerals, some in particular such as vitamin D, copper and zinc may have the potential to influence inflammatory status. This could be important in the context of bariatric surgery, as we know that typically the low-grade inflammation that occurs with obesity tends to resolve or improve with weight loss. It is possible however, that concomitant development of nutritional deficiencies could negatively impact the inflammatory improvement seen with bariatric surgery. However, this hypothesis is preliminary and has not been proven and the potential interrelationships are complicated. The interplay between trace element nutritional deficiency, inflammation and, to some extent, weight loss after bariatric surgery has been recently reviewed in the context of hematological disorders.[74] This review suggests that the interplay between micronutrient status and circulating adipose-derived cytokines could contribute to hematologic abnormalities and anemia after bariatric surgery through a series of complex interactions and needs to be investigated further.[74]

### *2.4.1: Copper*

The relationship between dietary and serum copper with inflammation is complex and not fully understood. Copper may contribute to both pro- and anti-inflammatory effects. The major copper containing protein in the blood, ceruloplasmin, is an acute phase plasma protein that may contribute to protection from inflammation and injury in various inflammatory states, e.g., inflammatory bowel disease.[75] However, in a large population based cohort study, serum copper concentrations were shown to be inversely related to unfavorable metabolic markers (e.g. circulating glucose, uric acid, total cholesterol, and LDL cholesterol) and positively associated with increased high-

sensitivity CRP (C-reactive protein), a marker of inflammation.[76] The impact of decreasing circulating copper and ceruloplasmin concentrations on inflammation in the long-term after RYGB has not yet been established.

However, it is well established that copper deficiency occurs following RYGB. An analysis of copper levels in 52 patients five years following RYGB showed that 3.8% had copper deficiency.[73] A retrospective chart review of 136 patients who were on average 33-months post-RYGB, found that 9.6% were copper deficient and a longitudinal study completed as part of the same analysis which followed 16 patients for 24-months post-RYGB found that plasma copper decreased by 10.1% at 24-months, and ceruloplasmin activity decreased by 18.6% compared with baseline.[77] Compared with baseline values, a statistically significant decrease in white blood cells was also observed at 6- and 24-months post-RYGB in this population.[77] The incidence of the development of copper deficiency in this same longitudinal population was 18.8%.[77]

Copper deficiency, if left to progress can have serious complications. A published case study documented severe acquired copper deficiency development in two individuals >10 years following RYGB.[78] Typical presentation of copper deficiency includes abnormalities in gait, anemia, and with severe copper depletion, neutropenia.[78] As is highlighted by these case studies, because copper is not typically measured as part of routine care following bariatric surgery, copper deficiency may progress unnoticed and numerous incorrect diagnoses may be tested before the true issue is identified. While

overt copper deficiency is relatively uncommon following RYGB, subclinical decreases in circulating copper and ceruloplasmin may be more common and could have the potential to impact the inflammatory, hematologic, and neurologic systems.

*2.4.2: Zinc*

Although it is more common following BPD/DS, zinc deficiency can and does occur following RYGB. The prevalence of zinc deficiency in a group of 52 subjects following RYGB was reported to be 15.4% and 21.2% at 48- and 60-months post RYGB respectively.[73] In the same study, the circulating zinc level was associated with the alimentary limb length resulting from the surgery.[73] One long-term study looking at deficiency at least 5 years after RYGB (average 6.9 years) showed that 40.5% were deficient in zinc at their last follow-up.[79]

Zinc plays an important role in many of the functions of the immune system and is essential for the development of neutrophils and natural killer (NK) cells.[80] Mild zinc deficiency, induced by restricting dietary zinc intake to 3-5 mg daily in healthy subjects, has been shown to impair cell-mediated immunity, IL-2 production, and decrease NK cell lytic activity.[81] Additionally, in mild zinc deficiency, IFN-γ was decreased, but IL-4, IL-6 and IL-10 expression was not altered.[80] Overt zinc deficiency has also been shown to increase susceptibility to disease and infection.[82]

Only one study has attempted to evaluate the changes in trace element (iron, zinc, and copper) status and relate those changes and their potential associations with

inflammatory markers after RYGB. In a cohort of 63 women (mean age 36.9 ± 9.2 years, BMI 43.8 ± 4.3 kg/m$^2$ who were evaluated at baseline and 6-months after RYGB, Rojas et al reported that hemoglobin, serum ferritin, the size of the rapidly exchangeable zinc pool (potentially a better measure of zinc status than plasma or serum zinc), and plasma copper decreased and leukocytes polymorphonuclear neutrophils (PMN) and high sensitivity CRP decreased post-RYGB.[83] However, plasma and hair zinc as well as zinc protoporphyrin increased 6-months after RYGB. The increased zinc protoporphyrin was slightly positively associated with PMN, $r = 0.32$.[83] These authors did not observe any significant associations between other measured markers of inflammation (including adiponectin, high sensitivity CRP and leukocytes) and any of the iron, zinc, and copper parameters that were measured.[83]

### *2.4.3: Vitamin D*

We have previously reported that in the first year following RYGB, serum vitamin D levels increased concomitantly with weight loss in many (but not all) individuals.[84] In the extended post-operative period, however, others have shown that vitamin D deficiency develops and becomes prevalent in as many as 60.5%[79] or 63%[85] of patients after an average of 6.9[79] and 4 years,[85] respectively. In recent years, large volumes of research have been published linking vitamin D levels to inflammation. Vitamin D's distribution includes adipose deposition.[84] Vitamin D appears to modulate macrophage inflammation and treatment of low vitamin D status may reduce adipose inflammation. In a recent study utilizing peripheral blood mononuclear cells and cultured

bone marrow derived macrophages from mice, within physiologic ranges, higher 25- and 1,25-dihydroxy vitamin D concentrations were shown to decrease TNF-alpha mRNA levels and decrease IL-6 in culture supernatants.[86] TNF-alpha is known to be a potential inhibitor of mitochondrial biogenesis and functions through a number of mechanisms including inhibition of endothelial nitric oxide synthase (eNOS).[87] Vitamin D may shift T cell profiles from Th1 toward Th2.[88,89] Vitamin D status has also been linked with the levels of a number of interleukins (including IL-2, IL-6, IL-8, IL-12), IFNγ and MCP-1.[90,91]

## 2.5: Vitamin D Status Following Bariatric Surgery: Implications and Recommendations*

### *2.5.1: Introduction*

The relationship between obesity and low levels of circulating 25-hydroxyvitamin D (25(OH)D) is consistently observed.[92] While this relationship is not yet well understood, what is clear is that obesity and vitamin D deficiency are inversely related and there is a high prevalence of vitamin D deficiency in obese individuals.[93,94] Individuals with extreme obesity who qualify for bariatric surgery[42] are frequently vitamin D deficient before[95–102] and after[103–105] surgery. Consequences of vitamin D deficiency include an increased risk of osteopenia and osteoporosis, muscle weakness and falls, and general overall pain and discomfort.[106]

Vitamin D deficiency traditionally has been diagnosed based on the classic symptoms of osteomalacia and rickets due to its role in bone health. While vitamin D as it relates to bone health after bariatric surgery has been reviewed previously,[101,107] there is growing interest in the non-bone functions of vitamin D that may have implications for obesity-related comorbidities. Emerging evidence suggests that higher than sufficient levels of serum vitamin D may be necessary to realize the non-bone-health-related benefits of vitamin D, but there is no current consensus on the definition of *optimal* vitamin D status.[108]

There is ongoing debate over the definition of vitamin D deficiency. In their recent update, the Institute of Medicine concluded that serum 25(OH)D ≥ 20 ng/ml is sufficient to cover the needs of 97.5% of the population, and overt deficiency occurs when serum 25(OH)D is < 12 ng/ml.[109] In contrast, the US Endocrine Society maintained the previous definitions of deficiency as serum 25(OH)D < 20 ng/ml, insufficiency as serum 25(OH)D between 21-29 ng/ml, and sufficiency as ≥ 30 ng/ml.[110] These discrepancies are the combined result of differing goals for each group and the large gaps that exist in the vitamin D literature. The controversy over what constitutes vitamin D deficiency has been addressed by both sides,[108,111] but a consensus definition has not been reached. For the purpose of this review we define deficiency as < 20 ng/ml and insufficiency as < 30 ng/ml, unless reporting research where a different definition was used, in that case, the authors' definition will be noted when it is available.

Currently, there are no evidence-based guidelines for optimal vitamin D dosing strategies after bariatric surgery. It is also not known if attaining and maintaining sufficient or even *optimal* vitamin D levels before and after surgery improves outcomes. In this review we focus on summarizing the results of studies reporting vitamin D deficiency before and after surgery as well as studies assessing treatment and dosing regimens for vitamin D deficiency in the bariatric patient population. The current guidelines for treatment and potential implications of vitamin D deficiency in individuals following bariatric surgery are also discussed.

### *2.5.2: The Potential Impact of Bariatric Surgery on Vitamin D Absorption*

Understanding how vitamin D levels are affected by bariatric surgery requires knowledge of the various procedures commonly performed. Bariatric surgical procedures can be narrowly classified as restrictive, malabsorptive, or as a combination of both depending on the traditional understanding of how the procedure promotes weight loss. Briefly, procedures that reduce the volume of the gastric reservoir and limit the transit of food are generally termed *restrictive* and procedures that prevent the absorption of nutrients are widely termed *malabsorptive*.[112,113]

The LAGB procedure and the VSG are typically considered restrictive while the RYGB and the BPD with and without DS are considered restrictive and malabsorptive. For the LAGB procedure, an inflatable band is secured around the top of the stomach allowing for variable restriction of the movement of food from the small gastric pouch into the remainder of the stomach and the duodenum. During the VSG procedure the stomach is resected longitudinally, preserving the antrum and the pylorus. The newly created gastric pouch has an approximate volume of < 100ml. During the RYGB a small gastric pouch, approximately 15ml, is created and connected to the jejunum via what is commonly termed the Roux limb. Gastric and pancreatic secretions as well as bile are secreted into the pancreatobiliary limb, which is anastomosed with the jejunum. Longer Roux limbs result in a greater degree of malabsorption following surgery. For the BPD and the DS procedures, 70% of the stomach is resected and much of the small intestine is bypassed. BPD can be performed with and without DS where the proximal duodenum is

anastomosed to the distal small intestine to create a short common channel. The malabsorptive component of the DS is reversible because no intestine is removed during the procedure. Considering the gastrointestinal rearrangement and/or restriction that occurs following these surgeries, nutritional deficiencies, including vitamin D deficiency are common in this patient population.

Vitamin D is endogenously produced after skin exposure to UVB light and can also be absorbed from dietary sources of either ergocalciferol (vitamin $D_2$) or cholecalciferol (vitamin $D_3$). The two forms of vitamin D do not have equal biological activity and may not be equally effective at maintaining vitamin D status.[114] At doses lower than 1000 IU, vitamin $D_2$ and $D_3$ appear equally effective; however, at doses higher than 1000 IU per day, vitamin $D_3$ appears to be significantly more effective at raising serum 25(OH)D concentrations than vitamin $D_2$.[114,115] Ingested vitamin D is incorporated into chylomicrons, which are absorbed into the lymphatic system and then enter the venous circulation. Decreasing the absorptive area of the small intestine and altering the delivery of pancreatic secretions and bile with bariatric surgery may therefore lead to decreased absorption of vitamin D.[116] The understanding of vitamin D absorption following bariatric surgery is complicated by the fact that a number of other factors beyond simple malnutrition likely contribute to the vitamin D deficiency in individuals with obesity.[92] These include, but are not limited to, the potential sequestration of vitamin D by adipose tissue,[117] the dilution of vitamin D in the presence of increased adiposity,[118] a lack of sun exposure, and a potential interaction between low-grade inflammation and

vitamin D levels.[119] Because of the aforementioned factors and due to the substantial weight loss and lifestyle changes that occur afterward surgery, it is difficult to tease apart the impact of bariatric surgery on vitamin D absorption.

There are limited data concerning vitamin D absorption before and after bariatric surgery. Aarts et al[116] measured the response of 14 women to an oral dose of 50,000 IU vitamin $D_3$ at 1-, 2-, 3-, and 14-days after dosing before and after RYGB.[116] The investigators noted an approximate 25% decline in post-operative absorption,[116] indicating that dose adjustments may be necessary in order to replete vitamin D after surgery. The anatomical changes resulting from each of the four most common bariatric surgeries suggest that the greatest impact on vitamin D absorption is most likely to occur following the malabsorptive surgeries. Unfortunately, there are no studies directly assessing the absorption of vitamin D after LAGB, VSG, or BPD/DS.

### *2.5.3: Prevalence of Vitamin D Deficiency Before and After Bariatric Surgery*

#### *Pre-Operative Vitamin D Status*

Low vitamin D status is prevalent in the pre-bariatric patient population.[95–102] The pre-operative vitamin D status of bariatric patients has been thoroughly reviewed by Compher et al[101] In their analysis of 14 studies (1566 patients), only one study[120] reported a mean pre-operative serum 25(OH)D concentration > 32 ng/ml.[101] More recently, in a sample of 115 women assessed for eligibility to undergo bariatric surgery, de Luis et al[102] found that 71.3% had serum 25(OH)D levels < 30 ng/ml and 26.1% had levels < 15

ng/ml.[102] Numerous other studies in Europe[96,97,99] and the United States,[84,98] including northern and more southerly latitudes over the range of seasons, have also shown deficiency in a majority of study participants before VSG[96,97] and RYGB.[96,98,99] It is clear from these data that individuals undergoing bariatric surgery are frequently vitamin D deficient or insufficient.

*Post-Operative Vitamin D Status*

Vitamin D levels have also been evaluated after various bariatric surgeries with conflicting results noted. The prevalence of deficiency after bariatric surgery is likely impacted by the type of surgery performed, among other factors.

Very few studies have assessed vitamin D status following LAGB. DiGiorgi et al[121] reported a nonsignificant trend towards increased serum 25(OH)D from baseline at 3-, 6-, 12-, and 24-months after LAGB.[121] Before surgery, 58% of individuals who received LAGB were vitamin D deficient, after 24 months, 33% remained vitamin D deficient.[121] Mean serum 25(OH)D for this patient population before and after surgery was not reported.[121] All patients were advised to consume 800-1200 IU vitamin D daily before and after surgery.[121] Similarly, Sinha et al[122] reported that serum 25(OH)D levels significantly increased from pre-LAGB to 9-12 months post-LAGB (20 ng/ml to 33 ng/ml, P=0.03). [122] These individuals were instructed to consume 400 IU vitamin $D_3$ per day after surgery, and some individuals were instructed to consume either 1200 IU vitamin $D_3$ or 50,000 IU vitamin $D_2$ depending on their pre-surgery vitamin D status.[122]

From the limited evidence available, it appears that vitamin D status may moderately improve following LAGB when supplementation is included as part of pre- and post-operative care.

Despite the increasing popularity of the VSG procedure, there is a paucity of studies specifically addressing nutritional deficiencies afterwards. Most of the studies that do report vitamin D status after VSG are more appropriately classified as treatment studies and will be discussed later.[99,123] Moizé et al[96] reported the vitamin D levels of 61 individuals following VSG.[96] They found that at baseline, approximately 93% of these individuals had serum 25(OH)D levels < 30ng/ml. Post-operatively, all individuals were prescribed approximately 1040 IU oral vitamin D per day from daily multivitamins and calcium supplements containing vitamin D. Compliance with supplementation was not recorded. Four years after surgery, the percentage of individuals with serum 25(OH)D levels < 30 ng/ml ranged between 66% and 85%,[96] indicating that for many individuals, vitamin D deficiency persists long after VSG.

Numerous studies have assessed vitamin D status after RYGB and inconsistent results between studies are evident. Beckman et al[84] studied a cohort of 20 women for 1-year after RYGB and found that serum 25(OH)D increased by 10 ng/ml from baseline.[84] At baseline, all serum 25(OH)D levels were below 30 ng/ml.[84] Individuals in this study were advised to consume 400-800 IU per day.[84] Conversely, Ybarra et al[105] reported no significant change in vitamin D levels approximately 36-months post-RYGB compared to individuals with obesity who had not undergone RYGB.[105] In addition, the mean post-

surgery serum 25(OH)D concentration was 13.5 ng/ml, which was not different from non-RYGB subjects, suggesting that vitamin D deficiency is prominent both before and after surgery. Individuals in this study were not taking supplemental vitamin D and vitamin D levels before RYGB were not reported. Studies with longer follow-up times also suggest that vitamin D deficiency persists after RYGB for some individuals.[103] Johnson et al[103] prospectively evaluated 266 RYGB patients. At baseline, mean serum 25(OH)D was 25 ng/ml and 41/266 individuals were reported to have levels below 30 ng/ml. The mean post-operative serum 25(OH)D levels were 34 ng/ml and 35 ng/ml at 1-year and 2-years post-surgery, respectively.[103] Study subjects were reported to consume a standard multivitamin three times per day (approximately 800 IU vitamin D per day) however compliance was not ascertained.[103] Carlin et al[124] also reported increased, but still suboptimal, vitamin D levels post-operatively.[124] These authors followed 60 women for 1-year after RYGB.[124] Each woman was randomly instructed to consume either 800 IU alone or 800 IU plus an additional 50,000 IU vitamin $D_2$ per day, compliance was assessed during patient interviews but not reported for each group overall.[124] At baseline, mean serum 25(OH)D was not different between groups (19.7 and 18.5 ng/ml for the 50,000 and 800 IU groups, respectively).[124] After 1-year, 19.2% of the women in the group receiving 50,000 IU per day were still vitamin D deficient despite having significantly increased vitamin D levels when compared to the 800 IU per day group (37.8 ng/ml vs 15.2 ng/ml, $P<0.001$).[124]

Vitamin D deficiency following BPD has been evaluated in a limited number of studies.[85,104,125] In a retrospective analysis of post-BPD patients, 73% of individuals had vitamin D levels < 30 ng/ml and 47% had levels < 20 ng/ml.[104] Indicating that over approximately 3-years vitamin D levels continued to decrease despite supplementation of 50,000 IU per day after surgery, though compliance was not measured.[104] Additional studies have reported that vitamin D deficiency is present in 63% of individuals undergoing BPD after four years,[85] and in 50% after a median of 32 months.[125]

Comparative studies of the impact of different bariatric procedures on post-operative vitamin D status are limited in number. Reports indicate that the incidence of nutritional deficiencies after VSG is generally comparable with, or less than, the incidence after RYGB.[113,123] Coupaye et al[99] recently reported on a cohort of 202 patients who underwent RYGB, 30 of whom were matched for age, gender, weight, skin color, and season with patients who underwent VSG.[99] Preoperatively, 91% of the entire cohort were vitamin D insufficient and 80% were deficient. Six months after surgery insufficiency and deficiency rates were 76% and 44% respectively.[99] Between the two groups of matched controls (RYGB v. VSG), serum 25(OH)D levels six months after surgery were not significantly different. The participants were only prescribed a multivitamin providing 200 IU to 500 IU vitamin $D_3$ daily, indicating that weight loss likely had a greater impact on serum 25(OH)D levels following surgery than supplementation.[99] In contrast, Gehrer et al[123] reported a lower incidence of vitamin D deficiency after VSG than RYGB. Before surgery 23% of patients were vitamin D

deficient, and following VSG and RYGB, 32% and 52%, respectively, were deficient (P=0.02).[123] The mean follow-up time was 24-months after surgery.[123] Individuals in both groups received no vitamin D supplementation unless deficiency was identified and both groups lost a similar amount of weight 1-year after surgery.[123] LAGB was compared with RYGB by DiGiorgi et al[121] and they reported that vitamin D deficiency decreased from 58% and 67% pre-surgery to 33% and 40% two years post-surgery in individuals undergoing LAGB and RYGB, respectively.[121] Given the range of results reported here, it is difficult to clearly ascertain the mechanisms driving vitamin D deficiency in this population.

*2.5.4: Vitamin D Treatment and Dosage Studies*

There are a number of other factors that might contribute to the variability in vitamin D deficiency status reported in the literature. These factors include the level of and compliance to supplemental vitamin D after surgery and the method used to assess vitamin D status.

Treatment studies of vitamin D repletion using standard dosing levels ranging from 200-400 IU[126,127] and 800 IU[124,128] have been reviewed previously; these intake levels are generally ineffective at positively impacting vitamin D status.[92] One of the more comprehensive studies worth revisiting here was conducted by Aasheim et al[129] These investigators studied 31 RYGB and 29 DS patients who were instructed to consume 400 IU vitamin $D_3$ per day and reported increased serum 25(OH)D levels at 1-

year after RYGB (P<0.001, baseline mean = 13 ng/ml) and decreased at 1-year following DS (P=0.059, baseline mean = 12 ng/ml).[129] These findings occurred despite self-reported higher vitamin D supplement use (approximately 400 IU per day) by the DS group: 89% reported taking vitamin $D_3$ 1-year after surgery compared with 74% of RYGB patients.[129] Results from this study and others that investigated standard treatment of 200-800 IU per day[124,126–128] for vitamin D deficiency indicate that this level of supplementation is likely to be inadequate to restore vitamin D levels after surgery.

Higher doses of supplemental vitamin D have also been evaluated after bariatric surgery.[100,130–133] Moore and Sherman[134] evaluated vitamin $D_3$ supplementation following RYGB (n=11) and VSG (n=12) and found that daily supplementation of 2000 IU vitamin $D_3$ three months following surgery reduced the prevalence of deficiency from 60.6% to 26.1% (P=0.005). They report that the response to supplementation was not different between RYGB and VSG.[134] Goldner et al[130] evaluated three levels of vitamin D supplementation in a prospective randomized pilot trial following RYGB.[130] They studied the efficacy of 800, 1200, and 5000 IU levels of supplementation (n=45) and found that 47%, 70%, and 70% of each respective group achieved 25(OH)D levels > 30ng/ml after 12 months.[130] However, even after 5000 IU per day for two years, only 78% of participants achieved serum 25(OH)D levels > 30 ng/ml, indicating that many patients may require even higher daily doses of vitamin D to achieve sufficiency in the years following surgery.[130]

Intramuscular injected ‘megadoses’ of vitamin D show some promise as adjunctive therapies for vitamin D deficiency following BPD. Einarsdóttir et al[133] demonstrated that 600,000 IU vitamin $D_3$ delivered intramuscularly to 29 individuals post BPD significantly raised serum vitamin D levels from baseline at 1.5- ($P<0.001$), 3- ($P<0.001$) and 6-months ($P=0.014$) post-injection, but not at 9- ($P=0.248$) and 12-months ($P=0.278$).[133] Mean serum 25(OH)D concentration was 25 ng/ml and 28.6% of subjects had vitamin D levels below 25 ng/ml at baseline.[133] No side effects or adverse events were reported during follow-up. Individuals in this study were also prescribed 1280 IU oral vitamin $D_3$ throughout the study, however compliance was not assessed.[133] The highest serum 25(OH)D level recorded was 64 ng/ml, indicating that this delivery method and level of supplementation did not raise serum 25(OH)D to levels outside of the normal range.[133] These authors concluded that because non-compliance with dietary supplementation of vitamin D is relatively high post-BPD, a once or twice yearly intramuscular depot of vitamin D could provide an effective treatment for deficiency.[133]

Another factor that may contribute to the variability of vitamin D status reported in the literature is the method used to evaluate serum 25(OH)D. There are many commonly used methods for measuring serum 25(OH)D. These include the competitive vitamin D protein binding assays (CPBA), immunoassays, high performance liquid chromatography (HPLC), and liquid chromatography-tandem mass spectroscopy (LC-MS/MS).[135] Most studies reviewed did not report the method of assay. Of those specifying the method, the vast majority used immunoassays.[105,122,124,129] Between

method variance has been widely observed and underscores the need for standardization of 25(OH)D assays.[135,136]Assays using antibodies or vitamin D binding proteins may be unable to distinguish between 25(OH)D and $25(OH)D_2$, or various metabolites of $25(OH)D_3$, leading to under and overestimation, respectively, when compared with LC-MS/MS.[137] LC-MS/MS has been chosen as a reference method by the National Institutes of Standards and Technology and also by the Centers for Disease Control and Prevention,[135] yet many studies still report serum 25(OH)D levels determined by immunoassays and other methods. For many researchers, LC-MS/MS analysis may be cost and time prohibitive. Expensive equipment, technical expertise, and relatively low throughput are just some of the disadvantages of LC-MS/MS that need to be overcome so that the method is more available to researchers. Consideration of the method used for analysis of vitamin D status may help to explain some of the contradictory evidence for vitamin D deficiency after RYGB. There needs to be a coordinated push for all researchers to adopt the same method for analysis of serum 25(OH)D, ideally LC-MS/MS, in order to allow for a true comparison between studies.

### *2.5.5: Current Recommendations for Treatment of Vitamin D Deficiency Before and After Bariatric Surgery*

Current recommendations from the US Endocrine Society advise individuals with obesity and those with malabsorptive syndromes to consume at least 6000-10,000 IU per day of vitamin D to treat deficiency (two to three times the level for healthy, normal weight adults) and to maintain serum 25(OH)D above 30 ng/ml.[108,110] They recommend

maintenance therapy of 3000-6000 IU per day once sufficiency is achieved.[108,110] The US Endocrine Society classifies these recommendations as weak, although based on high quality evidence.[110] While these guidelines were developed with a bariatric population in mind, as has been clearly demonstrated, daily supplementation even at two to three times the level recommended for healthy adults may be inadequate to maintain vitamin D sufficiency after bariatric surgery.

The AACE/TOS/ASMBS Bariatric Surgery Clinical Practice Guidelines recommend supplementation post-operatively with at least 3000 IU of vitamin D per day, with increasing dosage until a serum 25(OH)D level of > 30 ng/ml is reached (Grade A, based on randomized controlled trial data).[42] In cases of severe malabsorption they recommend vitamin $D_2$ or $D_3$ doses as high as 50,000 IU delivered one to three times per week up to daily, and more recalcitrant cases may require concurrent oral administration of calcitriol (Grade D, indicating that a 2/3 consensus of the task force could not be met for the recommendation).

The evidence for both of the aforementioned guidelines is quite limited because there have been very few randomized controlled trials of vitamin D dosing strategies. The US Endocrine Society cites two papers[138,139] as evidence in support of their recommendation for obese patients and patients with malabsorption.[110] However, neither of these papers are treatment studies of vitamin D repletion before and/or after bariatric surgery. The AACE/TOS/ASMBS guidelines are very comprehensive. However, the D

grade given for recommendation of up to 50,000 IU per day in cases of severe malabsorption indicates a low consensus among the task force members. These guidelines underscore the need for more research to develop evidence-based guidelines for repletion of vitamin D before and after bariatric surgery.

### *2.5.6: Implications and Clinical Outcomes of Vitamin D Deficiency after Bariatric Surgery*

While we know that vitamin D deficiency is prevalent before and after surgery, it is not clear what the potential impact of vitamin D deficiency or pre-surgery repletion would have on post-operative outcomes. Furthermore, this question becomes particularly important to consider in those individuals that develop significant post-operative complications (e.g. anastomotic leak, wound infection) and require intensive care. Vitamin D is thought to have wide-reaching effects that could potentially improve outcomes post-surgery. Active 1,25-dihydroxyvitamin D acts on various tissues throughout the body that express the vitamin D receptor, including adipose tissue, and may be responsible for up-regulating hundreds of genes that could impact multiple systems.[108] Vitamin D has been reported to positively influence cellular and immune functions including inflammatory pathways,[140] the renin angiotensin system and blood pressure regulation,[141] insulin sensitivity,[142] and other obesity related comorbidities.[108] Although observational studies support associations between vitamin D status and obesity-related comorbidities, there have not been enough long-term, randomized controlled trials to draw definitive conclusions.[143] If vitamin D does indeed have wide-

reaching effects throughout the body, the ramifications of vitamin D repletion have the potential for a profound impact on outcomes following bariatric surgery and may be particularly important in the setting of critical care.

In light of the expanding literature linking vitamin D with inflammation,[144] one recent study examined the impact of vitamin D deficiency on rates of post-RYGB infection. Quraishi et al[145] investigated the association between 25(OH)D levels and the risk of hospital acquired infections (HAI) after RYGB through a retrospective analysis of 770 patients. They found that the risk for HAIs was three-fold greater (adjusted odds ratio 3.1; 95% CI 1.3-6.9) for patients with serum 25(OH)D levels < 30 ng/ml when compared to patients with levels > 30 ng/ml.[145] What we do not know is whether repletion of vitamin D prior to surgery can have an impact on inflammation and decrease the risk of HAIs and complications following surgery.

With the continued trend towards increasing rates of obesity and bariatric surgery it follows that individuals with a prior history of bariatric surgery will constitute an increasing percentage of those requiring care for critical illness.[146,147] Furthermore, complications associated with aging including sarcopenic obesity are likely to be particularly problematic in individuals who have undergone bariatric surgery. These potential complications could be compounded by vitamin D deficiency in these individuals, given its potential to diminish muscle function and strength.[148] Vitamin D deficiency has indeed been correlated with decreased muscle strength and incidence of

falls in older adults.[148–151] Although it has not been the focus of current research on vitamin D and bariatric surgery, there is a pressing need for additional research to elucidate the role of vitamin D repletion in the management of muscle weakness and sarcopenic obesity in general, as well as in the critical care setting, in aging individuals with a history of bariatric surgery.

### *2.5.7: Research Needs and Future Directions*

The question of how to best treat vitamin D deficiency in a bariatric population remains unanswered. First, it is not known if repletion of vitamin D before bariatric surgery will have any impact on clinical outcomes. There are no randomized controlled trials to assess the effect of improving vitamin D status before surgery on post-operative clinical outcomes. Given vitamin D's roles in regulating the immune system, it seems plausible that improving vitamin D status before surgery could result in decreased rates of surgical site infections and complications. More research is needed to determine if supplementation with an aim of achieving sufficiency prior to surgery should be recommended.

Second, the question of how to determine optimal dosing regimens for repletion after each type of bariatric surgery, and how to adjust these regimens for long-term maintenance once sufficiency is achieved remains unanswered. Absorption and dosing studies are needed to establish specific guidelines for each type of bariatric surgery.

Third, a number of other potential routes of vitamin D administration exist that have yet to be tested in a bariatric population. A cursory search of the online retailer Amazon.com revealed numerous supplement companies who market vitamin $D_2$ and $D_3$ dietary supplements in patch, cream, and sublingual spray form. Very little research exists to corroborate the use of alternative routes of delivery of vitamin D. One recent small pilot study from Saudi Arabia demonstrated that daily topical application of 5000 IU vitamin $D_3$ to the skin of 48 healthy individuals for three months was successful in significantly raising serum 25(OH)D concentrations from 12 ng/ml to 38 ng/ml ($P=0.001$).[152] Routes of delivery that bypass the gastrointestinal tract should be investigated further particularly in individuals undergoing malabsorptive procedures.

Last, not only is the aging bariatric population of significant concern, the younger bariatric population consisting of adolescents has been understudied. Other nutritional complications, including vitamin D deficiency, in these young individuals are particularly concerning given the potential impact on growth and development. Furthermore, these individuals will present important challenges for the medical community as they mature, reproduce, and age. It is likely that vitamin D deficiency both in the immediate and long-term following bariatric surgery could have important implications for health outcomes.

A number of logistical considerations should be made when designing future research to address these important issues. These include but are not limited to: recording vitamin D intake from diet and supplements and estimating UVB exposure; assessing

compliance with supplementation regimens potentially with electronic methods; including controls and randomization when appropriate; and using a standardized and validated 25(OH)D assay method (LC-MS/MS).

### *2.5.8: Conclusion*

Although vitamin D deficiency is widely observed in individuals undergoing bariatric surgery, very little is known about post-operative changes in vitamin D absorption and other factors that may be driving vitamin D deficiency in these individuals. From the limited dosing studies available it appears that the DRI for vitamin D is inadequate for this patient population. It has been reported that doses of 5000 IU per day can be effective, but even 50,000 IU per day is not always effective at achieving vitamin D sufficiency in some individuals. Clearly, more research is needed to develop evidence-based guidelines for dosing specific to each type of bariatric surgery. Current guidelines recommend anywhere from 3000 IU-50,000 IU per day depending on the estimated severity of malabsorption. The clinical implications of vitamin D deficiency in these individuals are relatively unexplored; vitamin D insufficiency post-RYGB was reported to increase the risk for HAIs. Vitamin D's role in maintaining immunity and muscle function could have important clinical ramifications for the aging bariatric population, particularly in the critical care setting. Additional, well controlled studies are needed: 1) to evaluate the impact of vitamin D repletion before surgery on post-operative clinical outcomes; 2) to develop effective vitamin D repletion and maintenance dosing regimens and identify optimal routes of administration specific for each type of surgery;

and 3) to better understand the implications of vitamin D deficiency and optimal repletion in both young and aging bariatric populations.

## 2.6: The Current Study

In the short-term following RYGB significant changes in body composition occur. These changes include decreased FM and LST. The current literature indicates that in the long-term after RYGB weight regain tends to occur. It is not clear which body compartments are affected by weight regain after bariatric surgery. Changes in FM could have ramifications for inflammation and could be influenced by nutrient status. It is not known if field techniques for the assessment of body composition can be applied to the setting of long-term weight management after bariatric surgery to monitor these changes. An important question is whether we can monitor these changes with existing BIS technology.

In this dissertation the relationships between long-term body composition, nutritional status and inflammation are explored. In Chapter 3 the long-term changes of body composition and nutritional status are investigated in a small cohort of 5 women 8.5-years after RYGB. While the focus of this pilot study is primarily on body composition, nutritional status is also assessed, but only as it relates to the potential interplay between specific vitamins and minerals (namely: vitamin D, copper and zinc) and inflammation. In Chapter 4 an analysis of an advanced model for BIS assessment of body composition is presented with the goal of validating this model in comparison with DXA data from an NHANES dataset and a longitudinal dataset of women 1-year after RYGB. In Chapter 5, these studies are summarized and conclusions regarding their implications for future research are discussed.

# CHAPTER 3: LONG TERM FOLLOW-UP OF BODY COMPOSITION AND NUTRITIONAL STATUS FOLLOWING ROUX-EN-Y GASTRIC BYPASS SURGERY

### 3.1: Overview

**Background:** The Roux-en-Y gastric bypass (RYGB) has been the most commonly performed bariatric surgery over the past decade; it is traditionally classified as a restrictive and malabsorptive procedure, but hormonal changes also likely contribute to its success in inducing weight loss. Few data exist regarding the long-term impact of RYGB on nutritional status, body composition, and tissue level inflammation.

**Methods:** Five women from an original larger cohort were monitored pre-RYGB and 1.5-months, 6-months, 1-year and 8.5-years post-RYGB. Body composition was assessed using dual energy x-ray absorptiometry (DXA) and multiple dilution. Plasma, serum, and adipose tissue biopsies from surgery and 8.5-years post-RYGB were assessed for makers of nutritional status and inflammation.

**Results:** Compared to baseline, 8.5-year mean lean soft tissue (LST) was 11.9 ±5.6 kg lower (overall P=0.004) and 8.5-year LST was 4.4 ± 3.0 kg lower than 1-year post-RYGB (paired P=0.04). Fat free mass (FFM) decreased over the 8.5-year period by 12.6 ± 5.8 kg (overall P=0.004). Total bone mineral content (BMC) also decreased over the 8.5-year period from 3.0 kg to 2.3 kg (overall P=0.025). Unlike other tissue types, mean fat mass (FM) decreased after surgery from 75.4 ± 22.6 kg to 35.5 ± 21.5 kg 1-year post-RYGB (paired P=0.0004), but then increased by 8.6 ± 7.0 kg after 1-year (paired P=0.05). Loss of LST was moderately well correlated with loss of handgrip strength ($r$ = 0.64, P=0.0005). Vitamin D status increased over time (P=0.01). Tissue level inflammation measured by cytokines (MCP-1, IL-6, and IL-8), trended towards a decrease over time, but was not significantly different from time of surgery.

**Conclusion:** Improvements in vitamin D status and inflammatory status occur in good responders to RYGB. Continued decreases in LST, FFM, and BMC occur on the background of weight regain as FM. Losses of LST are correlated with decreased functional status measured by handgrip strength.

### 3.2: Background

The Roux-en-Y gastric bypass (RYGB) is a restrictive and malabsorptive procedure that reduces stomach capacity to approximately one ounce, bypasses part of the small intestine, and decreases nutrient absorption. Although overt macronutrient malabsorption is rarely reported, possible adverse outcomes of RYGB include protein deficiency, specific vitamin and mineral deficiencies, anemia, bone loss, abnormal fluid distribution, and excessive lean tissue loss.[153–156] While body composition has been measured by many groups in the first year after surgery,[39,46–49,157–159] few data exist regarding the long-term impact of RYGB on body composition. As might be expected, fat mass (FM)[39,46,49,158,159] and percent body fat[46,47,49,158] have been observed to substantially decrease after RYGB; however, fat free mass (FFM) also decreases after surgery, albeit to a lesser extent. The degree to which FFM is lost varies among individuals undergoing RYGB and is influenced by factors including dietary protein intake, exercise and mobility, and inflammation.[53,57,160,161] It is not well-studied what impact the long-term changes in body composition, particularly FFM, may have on muscle strength, function, and quality of life over the long-term in post-RYGB patients.

Within the FFM compartment is the intracellular water (ICW) and extracellular water (ECW), comprising the total body water (TBW). Abnormal fluid distribution between the ECW and ICW has been widely observed in the obese state. Individuals with obesity have been found to have a relative expansion of the ECW compartment, which is typically expressed as an elevated ECW/ ICW or ECW/TBW ratio.[38,40] For

example, the average ECW/ICW ratio of women with obesity (body mass index [BMI]: 48.8 ± 6.8 kg/m$^2$) has been reported to be 0.82 ± 0.17, while in lean individuals (BMI: 21.0 ± 2.0 kg/m$^2$) was reported to be 0.63 ± 0.06.[38,40] Other researchers have evaluated the expansion of ECW and overall fluid distribution in bariatric populations prior to surgery and found elevated ECW/ICW ratios, e.g. 0.80 ± 0.08,[162] 0.89 ±0.18[163] and 1.48 ± 0.57.[39] The obesity associated expansion of ECW does not appear to normalize after significant weight loss, with investigators reporting no change in the ECW compartment following weight stabilization over the first 1-2 years after bariatric surgeries including duodenoileal bypass,[40] gastroplasty,[162] and lap band.[163]

The relative expansion of ECW in individuals with obesity before and after RYGB has ramifications for body composition assessment because it causes violation of underlying assumptions inherent within the two-compartment measurement methods such as densitometry (e.g. air displacement plethysmography [ADP]) and deuterium dilution. Most notably, the assumptions that FFM is hydrated at 73%,[20] and that the body has a density of 1.1 g/ml[15] may not hold true in the obese and post-obese state, as observed by others.[38–40,162] Therefore, a combination approach to body composition assessment in populations undergoing massive weight loss may minimize these errors, and allow for a more complete assessment of long-term lean tissue changes following RYGB.

In addition to the changes in body composition, the restrictive and malabsorptive components of RYGB may contribute, in the long-term, to the development of rare

micronutrient deficiencies such as zinc and copper deficiency. These minerals are absorbed primarily in the duodenum and stomach and their absorption is decreased following RYGB.[71] Deficiency of micronutrients (including vitamin D) can persist following RYGB despite supplementation; poor vitamin D, copper, and zinc status could have ramifications in terms of inflammation.[73,75,76,82] The potential inflammatory implications of nutritional deficiencies after RYGB have not been explored.

Here we report the descriptive results of an uncontrolled longitudinal long-term follow-up pilot study to assess body composition using multiple methods, nutritional status (with particular focus on vitamin D, zinc and copper), and inflammatory changes in the plasma and adipose tissue as measured by cytokine content and mRNA gene expression before and after RYGB.

### 3.3: Subjects

As has been previously described,[4] women with Class III obesity (BMI $\geq$ 40 kg/m$^2$) who were planning to undergo RYGB surgery at the Weight Loss Management and Surgery Center at the University of Minnesota Health, were recruited for the original 1-year longitudinal study between 2005 and 2007.[4] The follow-up study of these original study subjects was completed during 2014 and 2015 and was approved by the University of Minnesota Institutional Review Board (IRB) and the Clinical and Translational Science Institute (CTSI). Returning subjects were compensated for their time. The 20 original subjects who attended at least three study visits as part of the original study were

re-contacted through an IRB approved mailed letter and asked to return to the Masonic Cancer Research Unit (MCRU) for one study visit. Individuals were excluded from the study if they (1) were on medications known to influence body composition (e.g. corticosteroids, anabolic steroids), (2) had a pacemaker or other internally-placed biomedical device, (3) had conditions associated with significant hydration changes, and/or metabolic disturbances, such as pulmonary hypertension, congestive heart failure, abnormal thyroid function indices, neoplastic disease, liver failure, renal failure, type 1 diabetes, and poorly controlled type 2 diabetes, or (4) were pregnant. The original cohort was limited to women to maximize the homogeneity of the sample given the significant differences in body composition between men and women; and because at the time of recruitment, only ~15% of the patients undergoing RYGB at our site were male.

## 3.4: Methods

### *3.4.1: Study Protocol*

Participants who were previously enrolled in the original study were re-contacted by mail and attended one additional study visit. The study visit began in the morning after a 12-hour fast. Participants remained fasted throughout all visit procedures.

### *3.4.2: Anthropometric Measurements*

Each participant's height was measured to the nearest 0.1 cm using a wall-mounted stadiometer (model S100; AYRTON, Prior Lake, MN) and weight was measured to the nearest 0.1 kg using a stand-on digital scale (model 5002, SCALE-TRONIX, White Plains, NY). Waist and hip circumference were measured following

standardized procedures as outlined in the Anthropometric Standardization Reference Manual.[164]

### *3.4.3: Total Body Water and Extracellular Water*

The day before the long-term follow-up study visit, participants were asked to avoid alcohol and vigorous exercise, and to maintain their usual fluid intake, limiting water consumption to no more than 250 ml water during the 2-hours just prior to testing. To measure TBW, after an 12-hour fast consisting of nothing by mouth except water, participants consumed 25 g of a 16% (wt/wt) solution of deuterium oxide, prepared following the method described in Trabulsi et al[165] (supplying 4 g of 99.9% $^{2}H_2O$ per pre-weighted dose, Sigma-Aldrich) in tap water, followed by 25 ml tap water rinse. To measure ECW, participants consumed 1g/kg of a 3% (wt/vol) sodium bromide solution (Fisher) in tap water followed by a 25 ml tap water rinse. Serum samples were collected for analysis of bromide enrichment at baseline, and 4- and 5-hours post dose. The 5-hour time point was used for data analysis. Urine samples were collected for analysis of deuterium enrichment at baseline, and 3-, 4-, and 5- hours post-dose. The 5-hour time point was used for data analysis. Participants were asked to void their bladders hourly during the study visit and they did not break their fast or consume any additional fluids throughout the 5-hour post-dose equilibration period. Urine samples were analyzed for deuterium enrichment by the laboratory of Dr. Dale Schoeller (University of Wisconsin, Madison, WI).[22,165,166] TBW was determined from the deuterium space after reducing it by 4% to correct for exchange with the non-aqueous compartment.[22]

Serum samples were analyzed by high-performance liquid chromatography for bromide by the laboratory of Dr. Jennifer Rood (Pennington Biomedical Research Center, Baton Rouge, LA).[167,168] The bromide dilution space was used to calculate ECW using the equation:

**Equation 3-1: ECW calculation**

$$\text{ECW} = [\boldsymbol{dose}/([\boldsymbol{Br}]_{\boldsymbol{5-hour}} - [\boldsymbol{Br}]_{\boldsymbol{Basal}})] \times 0.95 \times 0.9$$

where 0.95 is the Donnan equilibrium correction and 0.9 corrects for the intracellular distribution (including red blood cells).[22]

The original TBW and ECW data collected during the first year of weight loss following RYGB were used for comparison with the long-term follow-up data. For the first year of the study, we completed multiple dilution using a peripheral intravenous (IV) dosing protocol for deuterium and sodium bromide as has been previously reported.[45] For baseline, 1.5-months, 6-months, and 1-year post-RYGB, TBW was calculated from the average deuterium enrichment of the 3-hour and 4-hour urine samples. For the same four time points, ECW was calculated from the 4-hour post-sodium bromide infusion serum sample. For all time points, ECW and TBW were converted to kg by multiplying by 0.993 (the density of water at 37°C). Oral dosing should result in slower time to equilibrium than IV dosing for both deuterium and bromide, thus we extended the equilibration time by 1-hour.

*3.4.4: Body Composition by ADP*

Body composition was assessed by ADP using the BOD POD® (COSMED USA, Inc.; Concord, CA) located at the Center for Neurobehavioral Development (CNBD),

University of Minnesota and following the standard BOD POD® protocol included with the device's software. The BOD POD® was calibrated each day before use. Body weight was measured using the device's electronic scale that was calibrated bi-weekly. Participants wore tight fitting Lycra® or spandex® swimwear or lightweight sports bra and close fitting underwear and covered their hair with a tight fitting swim cap. Participants were instructed to remove all metal and jewelry before entering the machine. The device reports the average of two raw body volume measurements. The Siri model was used to estimate body composition.[15] When the participant was capable of doing so, thoracic gas volume (TGV) was measured using the device. TGV measurement was repeated until a merit value (<1.0) was obtained to signify compliance with the TGV protocol. For the one participant who was unable to complete the TGV measurement, the device-predicted TGV result was used. Calculations were performed by the BOD POD's software (version 5.4.0). Repeat measurements of body volume using BOD POD have been shown to result in an error equivalent to 0.8% body fat.[169]

*3.4.5: Body Composition by Dual Energy X-ray Absorptiometry*

As described by Levitt et al,[45] half-body and whole body DXA scans from the original study were performed on the GE Lunar Prodigy (GE Healthcare, USA). By the time of the long-term follow-up the CTSI had discontinued the use of the GE Lunar Prodigy, so long-term follow-up visits were measured as both half- and whole-body (when possible) scans on the GE Lunar iDXA (GE Healthcare, USA). Half-body scans from baseline, 1.5-months, 6-months, and 1-year post-RYGB were re-analyzed using the Lunar iDXA software. The right-side half-body scans were mirrored around the mid-line

to create a "whole" individual for analysis. For each original (baseline, 1.5-months, 6-months, 1-year) scan, study participants were scanned using an acrylic board to separate arm tissue from breast tissue as previously described.[45] Re-analysis of the isolated board using the new software indicated that it was interpreted by the iDXA as contributing the whole-body equivalent of 1.165 kg to FM and 0.365 kg to FFM. Thus, these values were subtracted from the results for mirrored body FM, LST, and FFM from the original scans. No corrections were made to the mirrored half-body scan for the one long-term follow-up visit where an acrylic board was not used. Precision of the iDXA instrument has previously been reported to be <1% for whole body bone mineral content[170] and 1.5% for whole body FM and LST measures[171] in individuals with extreme obesity.

*3.4.6: Resting Energy Expenditure (REE)*

At the time of metabolic rate measurement study participants had been fasted for approximately 15-hours. After resting quietly in a recumbent position for at least 30-minutes, measurement of REE was completed using a portable metabolic cart (TrueOne® 2400, Parvo Medics, East Sandy, UT). A trained technician completed the REE measurement following the standard CTSI clinical protocol. Briefly, after a 30-minute device calibration period during which time the subject rested quietly in a quiet, thermoneutral environment, a ventilated canopy was placed over the participant and gas exchange was measured for at least 20 minutes. The room was kept at a comfortable temperature throughout the measurement and the participant was instructed to not talk or sleep during the measurement period. The first 5 minutes of data were discarded. For analysis purposes results for each minute of testing for $VCO_2$, $VO_2$, RQ, and REE were

averaged for the time period from 6-20 minutes, which for all individuals had coefficients of variation ≤10%, interpreted as steady-state. REE measurements were available for all participants using the same protocol and device for the visit at 1-year post-RYGB.

### *3.4.7: Handgrip Strength*

Handgrip muscle strength was measured using a digital isokinetic hand dynamometer (Model T.K.K.5401, Grip D, Takei Scientific Instruments, Ltd., Japan). Handgrip strength was assessed with participants in a standing position. Participants were asked to keep their arm straight (elbow at full extension) and hanging by the side of their body. Participants were asked to squeeze the dynamometer with as much force as possible without changing their body position or leaning to one side. Two measurements were taken from the right and the left hand consecutively (R, L, R, L) and the four measurements were averaged. Handgrip assessment was completed at all visits of the original study following the same protocol.

### *3.4.8: 4-Component Model*

At the 8.5-year post-RYGB time point for which ADP was available to measure body volume, we calculated FM using the four-component model:[28,172]

**Equation 3-2: FM by 4-Component Model**

$$\text{FM}, \boldsymbol{kg} = \mathbf{2.7474} \times (\text{body volume}, \boldsymbol{l}) - \mathbf{0.7145} \times (\text{TBW}, \boldsymbol{l}) + \mathbf{1.4599}(\text{BMC}, \boldsymbol{kg}) - \mathbf{2.0503} \times (\text{body weight}, \boldsymbol{kg})$$

with body volume from ADP, TBW from deuterium dilution and bone mineral content (BMC) from DXA. FFM was calculated by subtracting FM from body weight.

*3.4.9: Adipose Biopsy*

At the follow-up visit, a subcutaneous adipose tissue biopsy was collected from each study subject. Briefly, under standard sterile technique and local anesthesia (1% lidocaine), an incision was created in the inferior periumbilical abdominal area and approximately 1 g of subcutaneous adipose tissue was obtained from each subject. The specimens were immediately frozen with liquid nitrogen in aliquots of 100-200 mg, and stored at -70°C until further study. At the time of the original surgery, for 4/5 subjects, a subcutaneous adipose tissue sample was collected as was previously described.[84] These adipose samples were stored at -70°C since collection.

*3.4.10: Serum Vitamin D and Plasma Micronutrients*

Fasting serum was collected in red-top gel vacutainer blood tubes. Fasting plasma was collected in sodium-heparin trace element free vacutainer blood collection tubes (Greiner Bio-One North America, Monroe, NC). Whole samples were allowed to clot for 30 minutes after collection and spun at 10,000 x g for 10 minutes to separate serum. Plasma samples were spun at the same time and speed. Serum and plasma were sent directly to the University of Minnesota Health-Fairview Labs for analysis of serum 25-OH vitamin $D_2$ and $D_3$ [25(OH)D] by liquid chromatography-tandem mass spectroscopy (LC-MS/MS), serum comprehensive metabolic panel, and plasma copper and zinc. Remaining aliquots of serum and plasma were frozen at -70 C. At each of the original study visits, serum 25(OH)D was assessed using the DiaSorin LIAISON total 25-OH-vitamin D assay method (DiaSorin, Stillwater, MN) as previously described.[84]

*3.4.11: Tissue and Plasma Cytokines*

Approximately 100 mg of subcutaneous adipose tissue from each subject (time of surgery, and 8.5-years post-surgery) was homogenized in 250 μl of ice-cold homogenization buffer (0.1% Triton X-100, 15 mM NaCl, 10 mM Tris-HCL with added protease inhibitors). After centrifugation for 15 minutes at 4°C at 3000 x g, the fat layer was discarded and the homogenate was centrifuged again for 20 minutes at 4°C at 14000 x g. The supernatant was immediately frozen on dry ice and stored at -70°C until processing. Tissue samples were normalized for protein content using the Bradford Assay. Plasma samples collected at time of surgery, 1-year post-surgery, and 8.5-years post-surgery were stored at -70°C until undiluted batch processing. Analysis of circulating plasma and subcutaneous tissue cytokines was completed by the Cytokine Reference Laboratory at the University of Minnesota using multiplex enzyme-linked imunosorbent assay (ELISA). All samples were run in duplicate. Cytokines assessed were: interleukin (IL)-1α, IL-1β, IL-1receptor agonist (ra), IL-2, IL-6, IL-8, IL-10, IL-12 (p40 and p70), interferon (IFN)-γ, monocyte chemoattractant protein (MCP)-1, and tumor necrosis factor (TNF)-α.

*3.4.12: Quantitative RT-PCR*

Expression of mRNA was measured by quantitative RT-PCR. Between 150-250 mg of subcutaneous human adipose tissue (from time of surgery and from long-term follow-up biopsy, four pairs total) was mechanically homogenized in Trizol reagent (Invitrogen Corp.). After treatment with deoxyribonuclease, cDNA was synthesized using an iScript cDNA synthesis kit (Bio-Rad Laboratories) using 1.5 μg of RNA.

Amplification was monitored with iQ SYBR Green Supermix and the MyiQ detection system (BioRad Laboratories). Data for overall changes in gene expression (pre-RYGB v. post-RYGB) for four sample pairs were analyzed using the -ΔΔCt method[173] and compared using student's t-test as -ΔCt values. All genes of interest were normalized to TATA box binding protein (TBP). Primer pairs for genes of interest were as follows: prostaglandin endoperoxide synthase-2 (COX-2) (sense primer 5'-CCC TTG GGT GTC AAA GGT AA-3'; antisense primer 5'GCC CTC GCT TAT GAT CTG TC-3'), cluster of differentiation (CD)40 (sense primer 5'-GCA GTG GGT GGT TCT GGA T-3'; antisense primer 5'-CTG GTC TCA CCT CGC TAT GG-3'), endothelial nitric oxide synthase (e-NOS) (sense primer 5'-GAC ATC TCC ATC AGG GCA G -3'; antisense primer 5'-TGA GTA TGA CGT GGT GTC CC -3'), integrin alpha M (ITGAM) (sense primer 5' GTG TCC TCA AGA GGA TAG TGA CAT T-3'; antisense primer 5'-CAG AGT ACT GCA TCA AAG AGA ACA A-3') and TBP (sense primer 5'-AGC GGT TTG CTG CGG TAA TC-3'; antisense primer 5'-ACT GTT CTT CAC TCT TGG CTC CTG-3').

### 3.5: Statistics

Statistical analyses were performed using SAS software, version 9.4 of the SAS system (SAS Institute Inc. Cary, NC, USA) and GraphPad Prism version 6.0 for Mac OS-X, (GraphPad Software, La Jolla California USA). Values are expressed as means ± standard deviations (SD), unless otherwise indicated. One-way analysis of variance (ANOVA) for repeated measures, with Geisser-Greenhouse correction for assumed

sphericity was used to determine significant differences in body composition measurements over time. Paired t-tests were used to compare between individual time points. A general linear model for repeated measures was used to determine the relationships between handgrip strength, LST, and age. For all tests, $P<0.05$ was considered significant. Pearson's correlation ($r$) and the limits of agreement from Bland-Altman (B-A) analysis are reported for comparisons between methods as is the coefficient of determination ($r^2$) when appropriate.

## 3.6: Results

### *3.6.1: Subject Characteristics*

Of the 20 Caucasian, female subjects who completed the original study, 10 expressed interest in returning to the study, 2/10 declined to participate in the follow-up study, 3/10 were lost to follow-up, and 5/10 individuals completed the follow-up study visit. Mean time to final long-term follow-up visit was 8.7 ± 0.3 years after surgery (see Table 3-1). All women were seen for the final follow-up between 8 and 9 years after surgery. Overall, 25% of the participants who completed the original study returned for the long-term follow-up visit. Mean age for these five women at time of surgery was 47.2 ± 10.9 years; at last follow-up visit, mean age was 56.2 ± 10.9 years. Most (4/5) women were post-menopausal at the time of final follow-up. One individual had diabetes (most recent Hemoglobin A1C of 7.6 mg/dl) and was taking insulin; all other individuals were non-diabetic. Fasting blood glucose was within normal limits (defined as >70 mg/dl and <99 mg/dl) at the follow-up visit for all participants. For the first three reported time

points (baseline, 1.5-months, and 6-months) post-RYGB, the individual with diabetes exhibited elevated fasting blood glucose levels.

### *3.6.2: Changes in Body Composition and Strength*

Table 3-1 shows the body composition of the 5 subjects at baseline (1-month before surgery), 1.5-months, 6-months, 1-year and 8.5-years after surgery. At each time point data were available for all five returning study subjects. Weight loss over the first year after RYGB was substantial. Mean weight loss to 1-year was 46.2 ± 10.2 kg which was significant compared with baseline (P=0.0005). Subjects gained 5.5 ± 6.7 kg of body weight back in the post-operative interval between 1-year and 8.5-years, although this weight gain was not significant (P=0.15). The relative greater weight loss success at 1-year post-RYGB is reflected in BMI changes over the 8.5-year period. BMI decreased at each time point until 1-year (from 48.8 ± 9.7 to 32.6 ± 9.2 $kg/m^2$, P=0.0003), and increased between 1-year and 8.5-years to 34.9 ± 8.8 $kg/m^2$ (P=0.0008).

**Table 3-1: Body composition and muscle strength over 8.5 years after RYGB surgery**

| | Baseline | 1.5-months | 6-months | 1-year | 8.5-years | P-value |
|---|---|---|---|---|---|---|
| Time relative to surgery, years | -0.09 ± 0.07 | 0.12 ± 0.02 | 0.51 ± 0.01 | 1.0 ± 0.005 | 8.7 ± 0.3 | - |
| Age, years | 47.2 ± 10.9 | 47.6 ± 10.9 | 48.0 ± 10.9 | 48.6 ± 11.0 | 56.2 ± 10.9 | <0.0001 |
| Weight, kg | 139.3 ± 35.9$^{a}$ | 125.4 ± 33.2$^{b}$ | 101.1 ± 32.2$^{c}$ | 93.2 ± 33.0$^{d}$ | 98.7 ± 30.2$^{cd}$ | <0.0001 |
| BMI, kg/m$^2$ | 48.8 ± 9.7$^{a}$ | 43.9 ± 9.0$^{b}$ | 35.3 ± 9.2$^{c}$ | 32.6 ± 9.7$^{d}$ | 34.9 ± 8.8$^{c}$ | 0.0001 |
| | | | | | | |
| DXA | | | | | | |
| Bone Mineral Content, kg | 3.0 ± 0.7$^{a}$ | 2.9 ± 0.6$^{a}$ | 2.7 ± 0.5$^{ab}$ | 2.7 ± 0.6$^{b}$ | 2.3 ± 0.6$^{c}$ | 0.025 |
| Lean Soft Tissue, kg | 63.5 ± 13.2$^{a}$ | 57.7 ± 12.3$^{b}$ | 54.1 ± 10.6$^{c}$ | 56.0 ± 10.6$^{b}$ | 51.6 ± 8.7$^{c}$ | 0.004 |
| Fat Free Mass, kg | 66.4 ± 13.9$^{a}$ | 60.6 ± 12.9$^{b}$ | 56.8 ± 11.0$^{c}$ | 58.6 ± 11.0$^{b}$ | 53.9 ± 8.0$^{c}$ | 0.004 |
| Fat Mass, kg | 75.4 ± 22.6$^{a}$ | 66.7 ± 23.0$^{b}$ | 44.1 ± 20.6$^{c}$ | 35.5 ± 21.5$^{b}$ | 44.1 ± 19.5$^{c}$ | <0.0001 |
| | | | | | | |
| Handgrip strength, kg | 31.9 ± 6.1$^{a}$ | 29.3 ± 5.8$^{b}$ | 29.1 ± 5.2$^{b}$ | 29.2 ± 5.5$^{b}$ | 26.2 ± 4.5$^{c}$ | 0.006 |
| | | | | | | |
| Multiple dilution | | | | | | |
| TBW, kg* | 47.8 ± 10.9$^{a}$ | 44.2 ± 8.8$^{b}$ | 42.5 ± 8.3$^{cd}$ | 44.3 ± 7.9$^{bd}$ | 39.5 ± 7.4$^{c}$ | 0.0021 |
| ECW, kg* | 21.6 ± 6.5$^{a}$ | 21.7 ± 5.6$^{a}$ | 20.9 ± 4.6$^{ab}$ | 22.3 ± 6.5$^{a}$ | 18.0 ± 4.4$^{b}$ | 0.06 |
| ICW, kg* | 26.2 ± 4.4$^{a}$ | 22.5 ± 4.3$^{b}$ | 21.6 ± 5.0$^{b}$ | 22.0 ± 3.4$^{b}$ | 21.5 ± 3.6$^{b}$ | 0.022 |
| ECW/ICW* | 0.82 ± 0.16$^{a}$ | 0.97 ± 0.20$^{ab}$ | 1.00 ± 0.26$^{ab}$ | 1.02 ± 0.28$^{b}$ | 0.83 ± 0.13$^{ab}$ | 0.21 |
| ECW/TBW* | 0.45 ± 0.05$^{a}$ | 0.49 ± 0.05$^{ab}$ | 0.49 ± 0.07$^{ab}$ | 0.50 ± 0.07$^{b}$ | 0.45 ± 0.04$^{ab}$ | 0.19 |
| | | | | | | |
| FFM hydration coefficient | | | | | | |
| TBW/DXAFFM* | 0.719 ± 0.03$^{a}$ | 0.731 ± 0.03$^{ab}$ | 0.748 ± 0.01$^{ab}$ | 0.757 ± 0.04$^{b}$ | 0.732 ± 0.03$^{ab}$ | 0.22 |

Abbreviations: Roux-en-Y gastric bypass, RYGB; BMI, body mass index; DXA, dual energy X-ray absorptiometry; TBW, total body water from $^{2}H_2O$ dilution; ECW, extracellular body water from bromide dilution; ICW, intracellular body water (ICW=TBW-ECW); FFM, fat free mass. Data are reported for n=5. All values are reported as mean ± SD. Overall P-value from repeated measures, one-way ANOVA, with Geisser-Greenhouse correction for assumed sphericity. Time points are baseline (pre-surgery) and 1.5-months, 6-months, 1-year, and 8.5-years after RYGB surgery. $^{a,b,c,d}$ Means that do not share a letter within a row are significantly different (P<0.05) by paired t-test. *TBW and ECW at long-term follow-up (8.5-years) using an oral dosing protocol not IV dosing protocol as with all other time points.

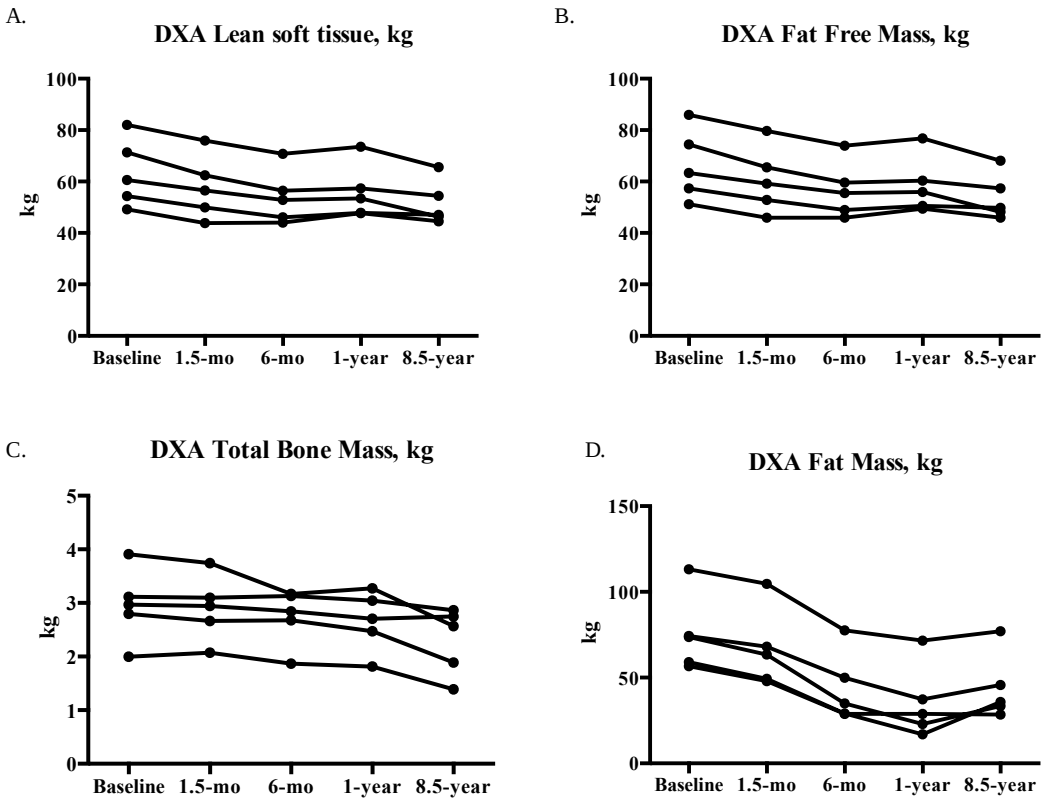
DXA data are reported in Table 3-1 as the result of mirrored analysis of half-body scans at each time point. At baseline, study participants were too heavy and/or large to obtain a whole body scan using the available standard sized DXA instrument; thus half-scans were performed initially as per others,[19,174] and for consistency, for all study visits up to 1-year post-RYGB. At the 8.5-year follow-up visit, both whole and half-body scans were obtained for the 4/5 individuals for whom it was possible, using the iDXA instrument. It has been previously reported that mirrored half-body scans are comparable to whole body scans using the iDXA.[19] To confirm validity of mirrored half-body scans in our small population, we compared mirrored half-body to whole-body scans from the long-term follow-up visit (n=4, for individuals with available whole-body and half-body scans). There were no differences between the whole-body and mirrored half-body scans for total BMC (P=0.98), total FM (P=0.94), total LST (P=0.91), and total FFM (P=0.91) and whole- and mirrored half-body scan were well correlated for BMC ($r$ = 0.99, P=0.0018), total FM ($r$ = 0.98, P=0.017), total LST ($r$ = 0.98, P=0.01), and total FFM ($r$ = 0.99, P=0.009).

The DXA data indicate that in this small sample, there was decreased LST, FFM, and BMC, which occurred on the background of weight regain, primarily as FM (Table 3-1, Figure 3-1). Compared to baseline, mean LST was 11.9 ± 5.6 kg lower by 8.5-years post-RYGB (overall ANOVA across time P=0.004; paired v. baseline P=0.009). In addition, 8.5-year LST was 4.4 ± 3.0 kg lower than 1-year post-RYGB (paired P=0.04). FFM followed a similar trend and decreased by 12.6 ± 5.8 kg over the 8.5-year period

(overall P=0.004; paired v. baseline P=0.008). BMC also decreased over the 8.5-year period from 3.0 ± 0.7 kg to 2.3 ± 0.6 kg (overall P=0.025, paired v. baseline P=0.03). Unlike the other tissue compartments, mean FM decreased after surgery from 75.4 ± 22.6 kg to 35.5 ± 21.5 kg 1-year post-RYGB (mean difference = 39.9 ± 8.32 kg, paired P=0.0004), but then increased by 8.6 ± 7.0 kg after 1-year (paired 1-year v. 8.5-years, P=0.05).

Results from TBW for the long-term follow-up were compared for 3-, 4-, and 5-hour urine collection time points by one-way ANOVA for repeated-measures. There was a difference between the three time points (P=0.009). Paired t-tests indicated that the 5-hour time point was higher than the 3- and 4-hour time points. The overall difference between 3- and 5-hours was only 0.53 l, thus, while significant the difference was not physiologically relevant and is below the precision estimates for the technique. In light of this, the 5-hour time point was selected for analysis purposes. For ECW calculated from serum bromide collected at 4- and 5-hours, the 4-hour was significantly lower than the 5-hour measurement by paired t-test (mean difference = 0.2 l, P-value =0.013). It has been previously demonstrated that bromide equilibrium may take up to 10-hours;[24] although due to logistical constraints this length of time is never utilized and instead, it is standard to use a 3-5 hour equilibration period in most studies. Thus, the 5-hour time point was selected for analysis purposes, in an effort to maximize the equilibration time.

**Figure 3-1: Changes in DXA measured tissue compartments after RYGB by individual including (A) lean soft tissue, (B) fat free mass, (C) total bone mass, and (D) DXA fat mass.**

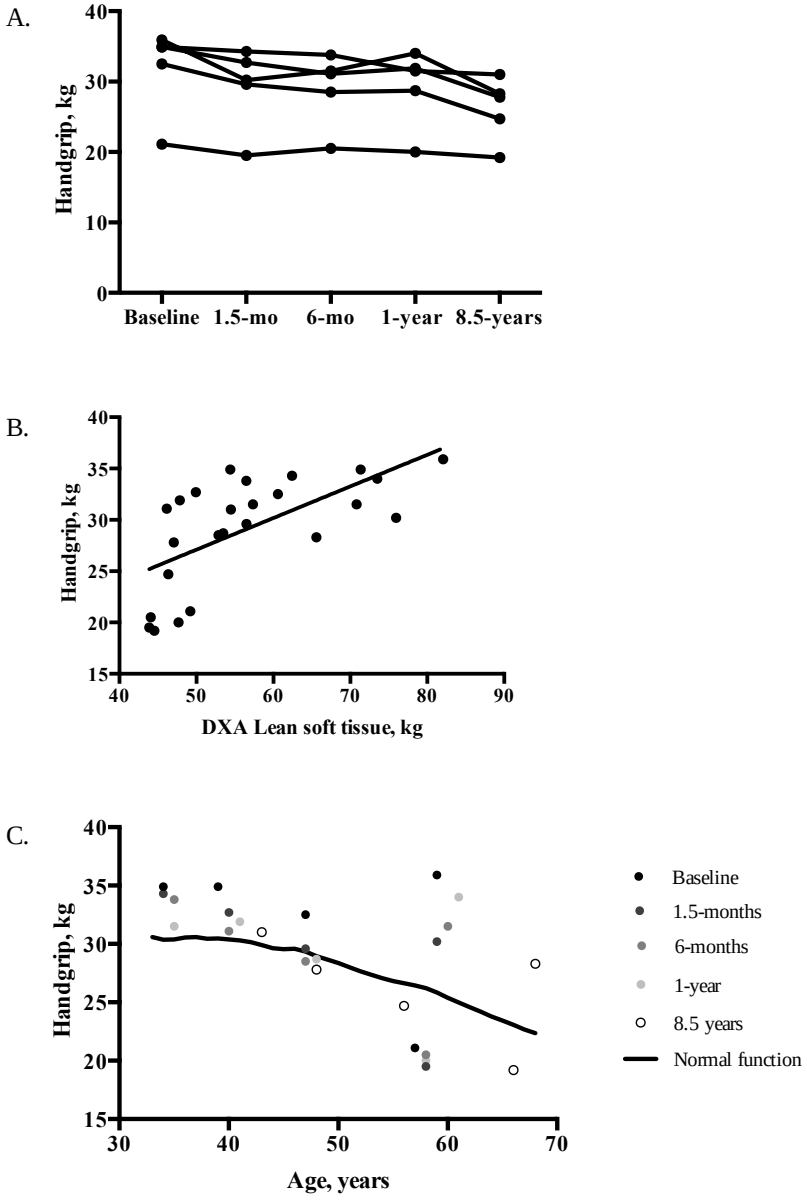



Abbreviations: RYGB, Roux-en-Y gastric bypass; DXA, dual energy X-ray absorptiometry; mo, month. Time points are baseline (pre-surgery) and 1.5-months, 6-months, 1-year, and 8.5-years after RYGB surgery. All data are reported as kg. See Table 3-1 for mean values and significance.

From Table 3-1, there was a significant decrease in TBW over the study period (overall P=0.0021) and a trend towards a decrease in ECW over the study period (overall P=0.06). The ECW/ICW and ECW/TBW ratios did not differ over the study period (overall P=0.21 and 0.19, respectively). The range of mean ECW/ICW values over the 8.5 time period was from 0.82 ± 0.6 to 1.02 ± 0.28, indicating that at all time points the mean ECW/ICW ratio remained elevated compared to what has been reported in the literature for lean individuals. The hydration of FFM (defined as TBW/DXA FFM) was not significantly different over time with weight loss (overall P=0.22). However, it is interesting to note that by 8.5-years post-surgery, the mean hydration of FFM was 0.732 ± 0.03, which was very similar to the reference hydration coefficient of 0.738.[20,21]

Interestingly, mean handgrip strength decreased over the 8.5-year period from 31.9 ± 6.1 kg of force to 26.2 ± 4.5 kg of force (overall P=0.006; paired baseline v. 8.5-years P=0.008) (Figure 3-2A). When including all points in the data set, and not accounting for the effect of each visit, this handgrip force was moderately well correlated with DXA LST ($r$ = 0.64, P=0.0005, $r^2$=0.41, n=25 pairs) (Figure 3-2B). To control for the effect of visits and aging over the course of the study, handgrip strength was assessed as a function of DXA LST, age, and visit. Fitting a general linear model for handgrip, LST, age, visit, and time since surgery, we found that only LST (P<0.0001) and age (P<0.0001) were significant contributors to the model. Visit and the time since surgery were not significant predictors of handgrip strength. The model containing LST, age, and visit accounted for 84.4% of the variation in handgrip strength in this small cohort. LST

**Figure 3-2: Changes in handgrip strength after roux-en-Y gastric bypass (RYGB) are correlated with changes in lean soft tissue measured by DXA.**




(A) Changes in handgrip force over time by individual, (B) correlation between lean soft tissue and handgrip force, (C) Handgrip force by age compared to manufacturer normal ranges by age. Abbreviations: DXA, dual energy X-ray absorptiometry; mo, month. Time points are baseline (pre-surgery) and 1.5-months, 6-months, 1-year, and 8.5-years after RYGB surgery. Normal handgrip strength values derived from handgrip device manufacturer (Model T.K.K., Grip D, Takei Scientific Instruments, Ltd. Japan).

is highly correlated with body weight in this sample ($r$ = 0.94, P<0.0001). To account for the contribution of body weight to LST in this sample we also assessed a model containing the percent of body weight from LST (where percent of body weight form LST is expressed as %LST=LST/weight), age, and visit. In this model both %LST and age were significant (P=0.003 and 0.0002, respectively), and the model accounted for 62% of the variation in handgrip strength.

Figure 3-2C shows the handgrip measurements by age for each visit plotted in comparison to the manufacturer's normal values for each age between 33 and 68 years (T.K.K.5401, Grip D, manual). At the final follow-up visit, 3/5 individuals were demonstrating below normal functional status for their age, and 2 of these 3 individuals had previously been classified as having normal handgrip force for their age.

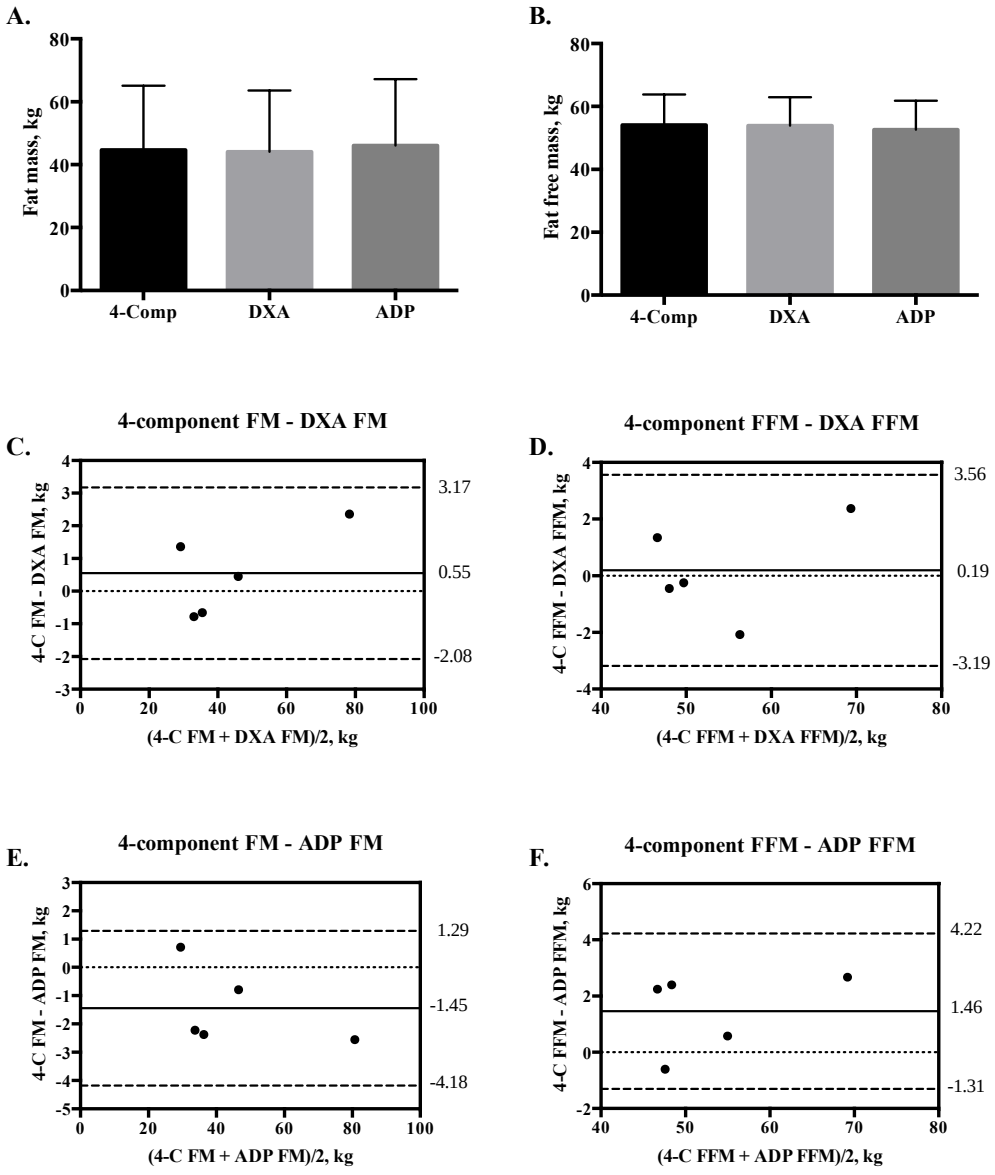
### *3.6.3: Comparison of FM by Multiple Methods at 8.5-Years Post-RYGB*

We calculated FM and FFM by the 4-component model, which utilizes body weight, BMC, TBW, and body volume and compared it to FM and FFM from the BOD POD® (calculated using the Siri model[15]), and FM and FFM from DXA (Figure 3-3A and B). There appeared to be no difference between FM by the 4-component model and DXA (mean difference = 0.54 ± 1.3; P=0.41), and the two methods were highly correlated ($r$ = 0.99, P<0.0001). Limits of agreement by B-A analysis were -2.09 to 3.17 kg, and the proportional bias was not significant ($r^2$ = 0.48, P=0.19) indicating relatively good agreement between methods (Figure 3-3C). There was also no apparent difference between FM by the 4-compartment model and BOD POD® (-1.5 ± 1.4; P=0.08), and the

two methods were highly correlated ($r$ = 0.99, P<0.0001). Limits of agreement by B-A analysis were -4.18 to 1.29 kg, and the proportional bias was not significant ($r^2$= 0.22, P=0.41) indicating relatively good agreement between methods (Figure 3-3E).

Similarly, there was no difference between FFM by the 4-component model and DXA (mean difference = -0.19 ± 1.7; P=0.82), and the two methods were highly correlated ($r$ = 0.99, P=0.001). Limits of agreement by B-A analysis were -3.19 to 3.56 kg, and the proportional bias was not significant ($r^2$ = 0.16, P=0.56) indicating relatively good agreement between methods (Figure 3-3D). There was also no detectable difference between FFM by the 4-compartment model and BOD POD® (-1.46 ± 1.4; P=0.08), and the two methods were highly correlated ($r$ = 0.99, P=0.0006). Limits of agreement by B-A analysis were -1.31 to 4.22 kg, and the proportional bias was not significant ($r^2$ = 0.14, P=0.54) indicating relatively good agreement between methods (Figure 3-3F).

**Figure 3-3: Comparison between methods for assessment of (A) fat mass, and (B) fat free mass 8.5 years post-roux-en-Y gastric bypass (RYGB) surgery.**




Abbreviations: 4-comp, 4-component model calculated from density by air displacement plethysmography (ADP) using the BOD POD instrument, total body water (TBW) by deuterium dilution, and total bone mineral content (BMC) by dual-energy X-ray absorptiometry (DXA) using the following equation: Fat Mass, $kg = 2.7474 \times (\text{body volume}, l) - 0.7145 \times (\text{TBW}, l) + 1.4599(\text{BMC}, kg) - 2.0503 \times (\text{body weight}, kg)$[28]

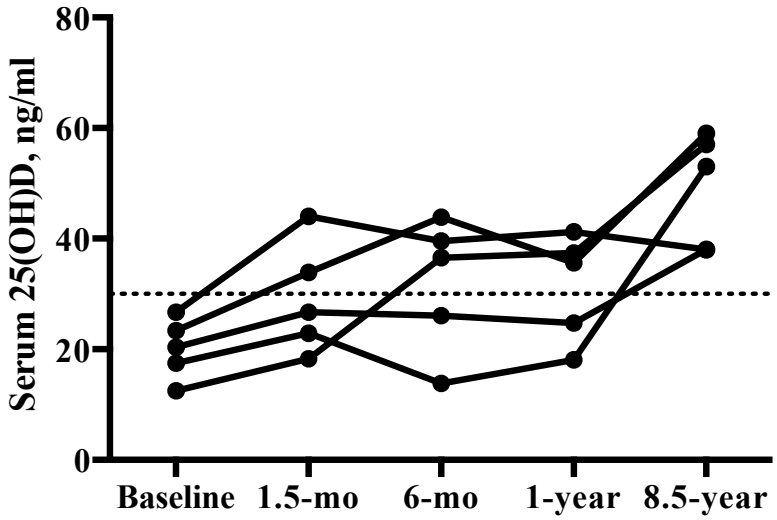
### *3.6.4: Changes in Vitamin D Status*

We have previously reported on the vitamin D status of these women as part of the original 1-year study.[84] We assessed the changes in vitamin D status in our small 5 individual cohort and found that vitamin D status increased over time post-RYGB (P=0.01) (Figure 3-4). At the time of surgery all 5 individuals in the study had serum 25(OH)D levels below 30 ng/ml, with mean 20.1 ± 5.5 ng/ml. At the 8.5 year follow-up all 5 individuals had serum 25(OH)D levels above 30ng/ml with mean 49 ± 10.27 ng/ml, indicating vitamin D sufficiency.[42,108] All individuals in this cohort were consuming vitamin $D_3$ supplements, with mean reported vitamin $D_3$ intake from supplements of 2500 ± 660 international units (IU)/day. Most of the women (4/5) reported that they were prescribed a multivitamin by their regular care provider and all of the women reported that they consumed at least one multivitamin per day. Most of the women (3/5) reported that they were consuming an additional prescribed vitamin $D_3$ supplement between 1200-3000 IU/day.

### *3.6.5: Micronutrient Status at 8.5-Years Post-RYGB*

At the long-term follow-up visit we measured copper and zinc using plasma collected in trace-element free tubes. These two micronutrients are not frequently measured post-operatively, but deficiencies have been reported to occur in the long-term after malabsorptive bariatric surgeries. We found that all individuals had normal zinc (mean 87 ± 30.5; range 67-137 ng/ml) and copper (mean 108.8 ± 8.8; range 98-121 ng/ml) status.

**Figure 3-4: Improved serum 25(OH)D status over time following roux-en-Y gastric bypass (RYGB). Dotted line indicates 30 ng/ml.**



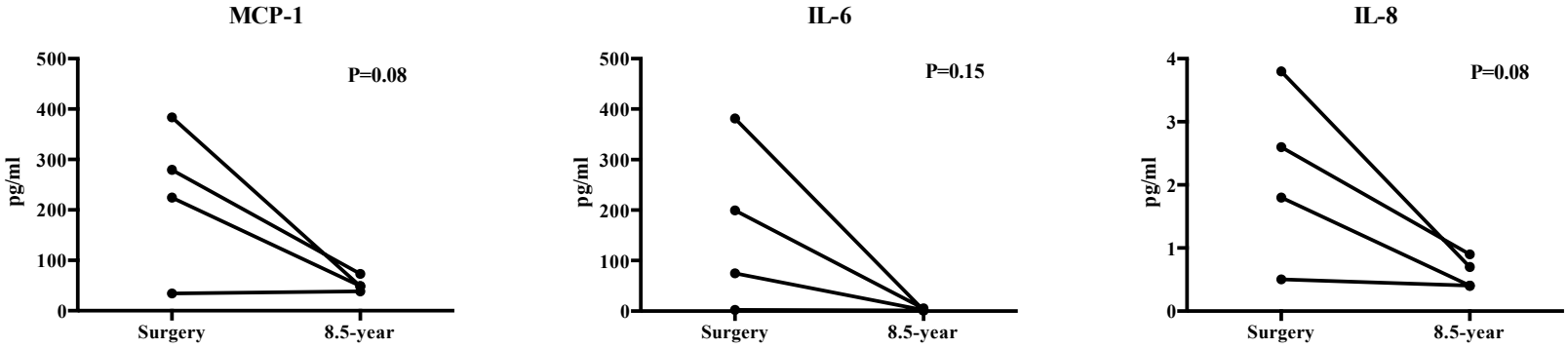

Abbreviations: 25(OH)D, 25-hydroxy-vitamin D; mo, month. Time points are baseline (pre-surgery) and 1.5-months, 6-months, 1-year, and 8.5-years after RYGB surgery.

*3.6.6: Long-Term Post-RYGB Inflammatory Status*

There were no significant differences in subcutaneous white adipose tissue expression of COX-2 (P=0.36, n=2), CD40 (P=0.85, n=4), eNOS (P=0.33, n=4), and ITGAM (P=0.55, n=4) based on comparison between surgery and 8.5-year samples. There were also no significant pre-/post-RYGB differences in adipose tissue cytokine levels. MCP-1, IL-6 and IL-8 tended to decrease in the long-term post-surgical subcutaneous adipose samples compared with those taken at the time of surgery, although these decreases did not reach statistical significance (P=0.08, 0.015 and 0.08, respectively) (Figure 3-5). TNF-α and IL-1ra were not changed in the long-term after surgery. IFN-γ, IL-10, IL-1β, IL-12p70 and IL-2 were not detectable in most tissue samples. IL-12p40 was detectable in 3/4 pairs and was not significantly different. IL-1α was detectable in 2/4 pairs and was not significantly different.

We were unable to detect changes between baseline, 1-year, and 8.5-years post-RYGB in plasma pro-inflammatory cytokines IFN-γ, TNF-α, IL-1α, IL-1β, IL-6, IL-8, IL-12p40, IL-12p70, IL-2, or anti-inflammatory cytokines IL-10 and IL-1ra. Plasma MCP-1 appeared to increase over the three time points assessed by one-way ANOVA for repeated measures (P=0.0002). Paired t-tests show that at 8.5-years after surgery MCP-1 was higher than baseline or 1-year. However this apparent increase, as well as the inability to detect changes in other cytokines, is most likely explained by the instability of plasma cytokines in long-term storage.[175,176]

**Figure 3-5: Cytokine expression in adipose tissue samples collected at time of Roux-en-Y gastric bypass (RYGB) surgery and 8.5-years post-RYGB.**




Abbreviations: MCP-1, monocyte chemoattractant protein-1; IL-6, interleukin-6; IL-8, interleukin-8.

## 3.7: Discussion

### *3.7.1: Body Composition and Handgrip Strength*

The results of our long-term assessment of body composition after RYGB agree with the findings of other researchers who have previously reported that significant changes in body composition accompany the weight loss following RYGB.[39,46–50,52,157,158] In our subjects, FM comprised ~84% of DXA total mass loss at 1-year post-RYGB. FM loss as a percent of weight loss has been reported to be between 73-80% at 6-months to 1-year after RYGB.[39,45–51] In our subjects, LST loss was ~16% of DXA total mass loss at 1-year post-RYGB. FFM loss has been reported to be between 18-25% of weight loss 1-year after RYGB;[39,46–48,51] and LST loss has been reported to be between 12-23% of weight loss 1-year after RYGB.[49,50] The majority of these assessments have been completed within the first two years following surgery. To the best of our knowledge, no other groups have assessed very long-term changes in body composition after RYGB. The most important finding from this study is the concerning trend of continued loss of LST in the background of weight regain, primarily as FM. At 1-year post-RYGB on average our subjects had 38% body fat and at 8.5-years that number had increased to 45%. Controlling for age, the loss of LST remains significant over time and is associated with a loss of functional strength as measured by handgrip force.

According to the device manufacturer, normal handgrip strength for 56-year old women is 26.6 kg, thus on average, at the long-term follow-up, the individuals in our study demonstrated slightly reduced functional status, with 40% of individuals classified

as below normal for their age at long-term follow-up when before surgery they were considered normal for their age. It is possible that weight regain and continued loss of lean tissue are related to a lack of physical activity. Reid et al have reported that in a cohort of 89 individuals 8.9 years post bariatric surgery with mean age of 50 years at time of follow-up do not meet current recommended step per day guidelines for physical activity.[58] More work is needed to determine if a lack of physical activity, independent of aging and other factors, is contributing to the apparent decrease in LST and functional strength.

Others have reported that in the long-term weight regain is a significant concern after bariatric surgery. In one cohort (n=300 with mean age at surgery=45.6 years) after a mean time of 6.9 years after surgery mean weight regain (as a percent of weight lost) was 23.4%.[66] In our cohort, mean percent weight loss was approximately 33% of total body weight while weight regain after 8.5-years was approximately 14% of weight loss and percent of weight regain from FM after 8.5-years was approximately 27% of FM lost, and 21% of weight lost.

*3.7.2: Markers of Inflammation After RYGB*

Macrophage infiltration of adipose tissue consists of pro-inflammatory (M1) and anti-inflammatory (M2) macrophages. M1 macrophages are recruited by pro-inflammatory cytokines related to expanded adipose tissue depots in obesity and once they infiltrate adipose tissue they secrete more inflammatory cytokines including MCP-1, TNF-α, IL-6, IL-1, IL-1β and IL-8.[177] We observed a trend towards decreased MCP-1,

IL-6 and IL-8, in long-term adipose biopsy samples which may indicate that over the 8.5-years after surgery, participants had less adipose infiltration from M1 macrophages compared to time of surgery, despite remaining classified as obese after weight loss. More research is needed to confirm this hypothesis. Interestingly, we were unable to detect any change in the monocyte/macrophage markers, CD40 and ITGAM, mRNA expression in our subcutaneous adipose tissue samples. CD40 is also expressed on human adipocytes, so this is by no means a perfect marker for macrophage infiltration of adipose tissue.[178]

Our finding of increased MCP-1 in plasma samples compared to baseline and 1-year does not agree with findings by others who have reported that plasma MCP-1 is higher in obese subjects compared to lean subjects and trends toward decreased levels after weight loss from RYGB (P=0.093).[179] We believe that our findings are directly related to the instability of plasma MCP-1 (and other cytokines) in long-term storage.[175] Future studies stemming from this pilot work should test cytokines in plasma samples as soon after collection as possible, and prior to freezing samples. Whenever possible for long-term studies where cytokine analysis by multiplex ELISA is planned or expected, an internal control should be created.[180]

For the other inflammatory genes of interest, COX-2 was only detectable at measurable levels in two sample pairs, which we might expect given that it may only be present during periods of active inflammation. In these two pairs there was no significant

difference between pre- and post-RYGB (P=0.36), but post-surgical expression was much lower than mean pre-surgical expression and the power to detect a difference was clearly limited by our sample size. Differences in eNOS expression, while also not significant (P=0.33), suggest that eNOS may have been lower in the post-surgical samples. An apparent trend towards decreased COX-2 and eNOS expression further supports the hypothesis that tissue level inflammation was decreased in the long-term post-surgical state.

### *3.7.3: Vitamin D, Copper, and Zinc Status*

Micronutrient status has not been studied in relation to changes in overall inflammatory status and body composition in the long-term following RYGB. The significance of this research is that zinc and copper deficiency have been implicated not only with anemia and neutropenia,[78] but also with neuromuscular dysfunction[71] and impaired immunity[73] making it relevant to monitor these essential trace elements in relation to long-term changes in body composition and immune function. Vitamin D has also become recognized as an immune modulator and is more closely monitored after surgery due to concerns with bone loss after surgery.[86,90]

We saw no evidence of copper deficiency in the long-term after RYGB. Our cohort reported good compliance with supplementation regimens, which likely contributed to their normal copper status. However it is well established that copper deficiency, while rare, can occur following RYGB. Others have reported that analysis of copper levels in 52 patients five years following RYGB showed that 3.8% had copper

deficiency.[73] A retrospective chart review of 136 patients who were on average 33 months post-surgery, found that 9.6% were copper deficient.[77] A longitudinal study following 16 patients for 24 months after RYGB found that plasma copper decreased by 10.1% at 24 months, and ceruloplasmin activity decreased by 18.6% compared with baseline.[77] Compared with baseline values, a statistically significant decrease in white blood cells was reported 6 and 24 months post-surgery and the incidence of the development of copper deficiency this population was 18.8%.[77] It is not reported if these studies use trace-element free tubes for plasma collection. Furthermore, a published case study documented the development of two cases of severe acquired copper deficiency at least 10 years following RYGB. In these cases, typical presentation includes abnormalities in gait, anemia, neutropenia associated with severe copper depletion.[78]

The relationship between dietary and serum copper with inflammation is complex and not fully understood. Copper may contribute to both pro- and anti-inflammatory effects. The major copper containing protein in the blood, ceruloplasmin, is an acute phase plasma protein that may contribute to protection from inflammation and injury in various inflammatory states, e.g., inflammatory bowel disease.[75] However, in a large population based cohort study, serum copper concentrations were shown to be inversely related to unfavorable metabolic markers (e.g. circulating glucose, uric acid, total cholesterol, and LDL cholesterol) and positively associated with increased high-sensitivity CRP, a marker of inflammation.[76] At 8.5-years post-surgery all individuals in

our study had normal copper levels; therefore, we cannot draw conclusions about the potential inflammatory effect of copper in this cohort.

We saw no evidence of zinc deficiency in our cohort, and no evidence of any relationship with inflammatory status. Overt zinc deficiency has also been shown to increase susceptibility to disease and infection.[82] While more common following biliopancreatic diversion, zinc deficiency can occur following RYGB. The prevalence of zinc deficiency in a group of 52 subjects following RYGB has been reported to be 15.4% and 21.2% at 48 and 60 months post-RYGB, respectively.[73] In the same study, the circulating zinc level was found to be determined by the alimentary limb length resulting from the surgery.[73] One long-term study looking at deficiency at least 5 years after RYGB (average 6.9 years) showed that 40.5% were deficient in zinc at their last follow-up.[79]

In our cohort all individuals had normal zinc status at their long-term follow-up. We do not have data for their baseline zinc status, though as with copper, it is likely that at baseline, zinc status was normal as zinc deficiency is exceedingly rare in the general population. Therefore we do not think that zinc status in our cohort was associated with any inflammatory changes. Zinc plays an important role in many of the functions of the immune system and is essential for the development of neutrophils and natural killer (NK) cells.[80] Mild zinc deficiency, induced by restricting dietary zinc intake to 3-5 mg daily in healthy subjects, has been shown to impair cell-mediated immunity, IL-2

production, and decrease NK cell lytic activity.[81] Additionally, in mild zinc deficiency, IFN-γ was decreased, but IL-4, IL-6 and IL-10 expression was not altered.[80]

We have previously reported that in the first year following RYGB, serum 25(OH)D levels increased concomitantly with weight loss in many (but not all) individuals.[84] However, others have observed that vitamin D deficiency develops in as many as 60.5%[79] or 63%[85] of patients after an average of 6.9[79] and 4 years,[85] respectively. In recent years, many studies have been published linking vitamin D levels to inflammation. Vitamin D's distribution includes adipose deposition.[84] Vitamin D appears to modulate macrophage inflammation and treatment of low vitamin D status may reduce adipose inflammation. In a recent study utilizing peripheral blood mononuclear cells and cultured bone marrow derived macrophages from mice, within physiologic ranges, higher 25- and 1,25-dyhydroxy vitamin D concentrations were shown to decrease TNF-alpha mRNA levels and decrease IL-6 in culture supernatants.[86] TNF-alpha is known to be a potential inhibitor of mitochondrial biogenesis and function through a number of mechanisms including inhibition of endothelial nitric oxide synthase (eNOS).[87] Vitamin D may shift T cell profiles from Th1 toward Th2.[88,89] Vitamin D status has also been linked with the levels of a number of interleukins (including IL-2, IL-6, IL-8, IL-12), IFNγ and MCP-1.[90,91] To the best of our knowledge, vitamin D deficiency in relation to inflammation post RYGB has not been studied. We saw no evidence of vitamin D deficiency in our cohort. Vitamin D status continued to increase throughout the study. At the final follow-up visit, all 5 of the returning subjects had vitamin D levels >30

ng/ml and there was no indication that vitamin D levels were associated with any changes in circulating or tissue level cytokines, although some subcutaneous adipose tissue cytokines did show a decreasing trend. More work is needed to determine if improved vitamin D status after RYGB is associated with tissue-level inflammatory improvement after RYGB.

### 3.8: Limitations

We recognize that there are a number of limitations with our study. This long-term follow-up study had a small sample size and was not powered to detect differences in small changes in inflammatory makers or gene expression or to detect differences in methods for comparing body composition (i.e. between 4-component model and DXA/BOD POD®). This pilot study was partly completed to determine the feasibility of long-term in-depth measurement of changes in body composition and inflammation. We found that between 8- and 9- years post-RYGB, only 25% of patients who completed the original study were willing to return. Future long-term studies in similar populations should consider the potential for very low return rates when powering studies.

Our study did not incorporate any male subjects. It is possible that changes in body composition for male subjects after RYGB are different from those seen in female subjects. There was also an inherent bias in our study sample. We do not know anything about the long-term weight loss success in the 15 women who did not return for follow-up, and it is possible that our return cohort was self-selected to include individuals who

had lost and maintained their body weight since the original study ended. Clearly, the cohort for this study was successfully able to lose and maintain their weight loss and all were compliant with supplementation regimens as evidenced by normal plasma vitamin and mineral levels. Finally, in any future assessments of the relationship between lean tissue and functional status it will be important to control for dietary protein intake and physical activity.

Given the long time between original study visits and the long-term follow-up a number of methodologies used were different. In particular, oral dosing was used in place of IV dosing for deuterium and bromide. We have attempted to account for these differences by using a 1-hour later time point for analysis of both deuterium and bromide than the original study. Serum 25(OH)D was assessed by immunoassay at the time points throughout the first year and by LC-MS/MS at the final time point. It has been reported that the DiaSorin LIAISON immunoassay method has good agreement with LC-MS/MS with concordance correlation coefficient (CCC) of 0.95, and *r* of 0.95.[181] The mean bias of the LIASON compared to LC-MS/MS is reported as 0.2 ng/ml.[181] Therefore, we have confidence that these two methods are close enough to be used for the purposes of our comparisons over time.

### 3.9: Conclusions

The continued loss of lean mass during the process of weight regain is a concerning trend in this population. This combined with the significant relationship

between loss of functional status as measured by handgrip strength and LST while controlling for age further indicates that in the long term there may be physiologically important loss of muscle mass and function in this population. On the positive side, we saw an increase in serum 25-OH-D and a trend towards improved tissue level inflammatory status. Clearly there is a tradeoff here; on the one hand we saw these women improve their vitamin D and inflammatory status (and at least in one case, metabolic status/blood glucose control), while on the other hand we saw that in the long-term there appeared to be a loss of functional status. More work is needed to control for factors including protein intake and activity level in a larger cohort, to determine the cause of lean tissue loss, and to demonstrate if it is reversible with exercise and lifestyle intervention.

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# CHAPTER 4: EVALUATION OF NEW ADVANCED BIOIMPEDANCE SPECTROSCOPY MODELS FOR MEASURING BODY COMPOSITION IN HEALTHY INDIVIDUALS (NHANES 1999-2004) AND THOSE UNDERGOING MASSIVE WEIGHT LOSS FOLLOWING ROUX-EN-Y GASTRIC BYPASS SURGERY

## 4.1: Overview

**Background:** Bioimpedance spectroscopy (BIS) devices utilize biophysical modeling to generate body composition data. The addition of body mass index (BMI) to modified Xitron-Hanai-based mixture equations improved BIS estimates (Moissl et al. Physiol Meas 2006). A model for distinguishing excess fluid (ExF) from normally hydrated lean (NH_LT) and adipose tissue (NH_AT; Chamney et al. AJCN 2007) may further improve BIS estimates. We aimed to validate a BIS approach based on the Moissl-Chamney models for determining fat mass (FM) in healthy individuals (NHANES) and for measuring FM changes in individuals undergoing massive weight loss.

**Subjects/Methods:** From the NHANES 1999-2004 dataset, we obtained BIS data (Hydra 4200, Xitron Technologies) for 5740 adults (2744 F, 2996 M; BMI 26.7±5.4 $kg/m^2$). We calculated extracellular (ECW) and intracellular water (ICW) by using the BMI-corrected mixture equations, then applied the ECW and ICW values to the Chamney equations to generate NH_LT, NH_AT and FM. and compared values for FM and NH_LT to dual-energy X-ray absorptiometry (DXA) FM and lean soft tissue (LST) for method validation by BMI category. For more in-depth validation, we separately generated longitudinal BIS data (Hydra 4200) from women with obesity before (BMI 46.6±6.8 $kg/m^2$, n=25) and 1-year following (BMI 31.6±6.3 $kg/m^2$, n=15) Roux-en-Y gastric bypass (RYGB) surgery. In both datasets, method agreement for FM by BIS and DXA was evaluated by correlations, paired t-tests, root mean square error (RMSE), Bland-Altman (B-A) plots, and concordance correlation coefficients (CCC).

**Results:** DXA and BIS-measured FM (adjusted for BMI) was well-correlated in healthy adults ($r$ = 0.96, P<.0001, CCC=0.93, RMSE=3.5 kg, n=5740), pre-RYGB ($r$ = 0.93, P<.0001, CCC=0.81, RMSE=6.47 kg, n=25) and at 1-year post-RYGB ($r$ = 0.98, P<.0001, CCC=0.86, RMSE=3.45 kg, n=15). Although mean FM by the two methods was different in the healthy subjects and at both time points in the RYGB subjects, measures of *change* in FM post-RYGB were not different between methods (35.6±8.9 vs 35.2±9.2 kg, BIS vs DXA, n=15) and were in reasonably good agreement ($r$ = 0.84, P<.0001, CCC=0.84, RMSE=4.99 kg), although the B-A analysis revealed several outliers (-9.6 to 10.3 kg). Interestingly, when evaluating the loss of lean tissue over the one-year follow-up period, our 15 subjects lost an average of 11.5±10.1 kg FFM by DXA, 9.4±2.5 by BIS BMI adjusted FFM and 11.6±10.1 kg of LST by DXA, but only 1.3±2.5 kg of NH-LT by BIS, suggesting that the LST loss was almost entirely adipose tissue water, assuming that the Moissl-Chamney model was valid in these subjects based on FM comparisons.

**Conclusions:** Taken together, these findings suggest that the incorporation of Moissl-Chamney type models into BIS approaches is an advancement that may ultimately prove groundbreaking for assessing and monitoring human body composition, with additional refinements in resistivity and other constants specific to the patient population of interest. Its ability not only to differentiate ICW from ECW, but also the potential to more specifically monitor lean tissue in the clinical setting is particularly promising.

## 4.2: Background

Bioimpedance spectroscopy (BIS) involves the application of biophysical modeling algorithms to bioimpedance data measured across a spectrum of frequencies (e.g. from ~5 to 1200 kHz, using data from 50 or more frequencies) in order to generate body composition data. Simply put, at low frequencies (e.g. approaching theoretical 0 frequency), the current produced by a BIS device is conducted only by extracellular water (ECW), due to the capacitance effect of cell membranes and tissue interfaces. At relatively high frequencies (e.g. approaching theoretical infinity frequency), BIS devices measure intracellular water (ICW) as the capacitive property is lost, allowing the current to pass through cell membranes and tissues and thus quantifying both ICW and ECW (i.e. total body water, TBW). The bioimpedance data are fit to the Cole model[30] using nonlinear least squares curve fitting; the semicircle formed by plotting resistance (R) and reactance (X) values is extrapolated to reach the x-axis in order to determine R at zero ($R_0$, also called $R_E$) and infinity ($R_\infty$). From these, $R_I$ can be calculated, representing the resistance related to the ICW. Cell membrane capacitance ($C_M$) is also calculated in the model. Cole model terms can be applied to the Xitron-Hanai-based[31,32] mixture equations to determine ECW and ICW; these equations are based on the conductivity of suspensions[33] and consequently can be used in conjunction with the presumed conductivity and resistivity of the different body compartments (i.e. ECW and ICW).

These equations incorporate several constants including intra- and extra-cellular apparent resistivity, shape factor, and body density. The most commonly utilized

apparent resistivity constants are the ones published by De Lorenzo et al;[31] these predominate in the literature and are programmed into the software accompanying the Hydra 4200 BIS device. The apparent resistivity constants for the intra- and extracellular compartments that are applied to the mixture equations require the assumption that the resistivity of all tissue types is constant, however, it has been shown that these constants may vary with adiposity, and the errors in ICW and related lean tissue compartments increase with increasing adiposity.[34–36] Although the use of obesity-specific resistivity constants did not significantly improve BIS estimates in individuals with extreme obesity,[34,35] better success was achieved by adjusting ECW and ICW estimates for body mass index (BMI).[182] Moissl et al[182] demonstrated that the addition of BMI to modified Xitron-Hanai-based mixture equations substantially improved BIS estimates at the extremes of BMI.[182]

BIS devices hold promise for bedside assessment in the clinical setting, as they provide a cost-effective, non-invasive technique which can be used for repeated measures with minimal risk to the individual.[29] They offer the potential for a more individualized, flexible approach to body composition assessment because of the fact that data are collected over an entire spectrum of frequencies and then fit to a mathematical, biophysical model (i.e. the Cole model) of human tissue; these qualities set BIS apart from the more traditional single- and multiple-frequency bioelectrical impedance analysis (SF-BIA, MF-BIA) approaches which rely on statistically derived, population-specific prediction equations. In particular, BIS offers the unique opportunity to individually

evaluate ECW and ICW, which would be particularly advantageous in individuals with acute or chronic disease who may have abnormal fluid distribution and edema. From ECW and ICW estimates, fat mass (FM), fat free mass (FFM), and other lean tissue body compartments can be calculated. Chamney et al have described a novel three-compartment whole-body model for distinguishing excess fluid (ExF) from normally hydrated lean (NH_LT) and normally hydrated adipose tissue (NH_AT) which may further improve BIS estimates.[183] This three-compartment model, based upon dilution, DXA and cadaver data, has been shown to have clinical utility for the assessment of excess fluid and tailoring clinical management in large scale studies of individuals undergoing dialysis.[184,185]

Dual energy X-ray absorptiometry (DXA) is frequently utilized as a reference method for comparison with BIS, particularly for FM and FFM measures. DXA typically produces photons at 40 and 70 keV, which pass through tissues at rates depending on their elemental composition.[14] DXA output allows for visualization and analysis of the separate tissue types. While DXA has its own limitations and sets of assumptions, it is well regarded as a method to accurately assess bone mineral content (BMC), FM, FFM, and non-bone lean mass, termed lean soft tissue (LST). Height, weight and width limitations have historically restricted the use of DXA to those individuals who are mobile and who meet the physical size specifications required to use the machine. However, newer machines are capable of measuring larger individuals.

The application of BIS data to Moissl BMI-corrected Xitron-Hanai-based mixture equations to generate the ECW and ICW values needed to calculate the Chamney 3-compartment model would potentially allow for a more specific evaluation of lean tissue by evaluating the NH_LT, accounting for excess fluid that can accumulate in both adipose and lean tissue compartments in clinical populations with fluid overload (e.g. individuals on dialysis, with extreme obesity, or with acute or chronic illness). The measurement of FM at the bedside would also be highly advantageous to the clinician interested in monitoring individuals undergoing weight loss interventions. Because we have no directly comparable reference compartment using DXA to validate the NH_LT compartment, we can evaluate it indirectly through the comparison of FM measures. Thus, we aimed to validate a BIS approach based on the combination of the Moissl-Chamney models for determining FM in healthy individuals from an NHANES data set compared to DXA measures, and for determining FM changes in individuals undergoing massive weight loss following Roux-en-Y gastric bypass surgery (RYGB).

## 4.3: Subjects

### *4.3.1: NHANES 1999-2004 Dataset*

Observations were extracted from the NHANES 1999-2004 datasets for both DXA and BIS data. BIS data were only available for individuals aged between 8 and 49 years. Body composition is heavily influenced by age, growth, and stage of development and estimating body composition in children may require specific equations incorporating child-specific resistivity, shape and body density constants; therefore, we restricted our

analysis to data from adults between the ages of 18 and 49 years. After exclusion of missing data and outliers the final sample consisted of 5740 observations.

### *4.3.2: Longitudinal Dataset*

Data were gathered from women with extreme obesity before (mean BMI 46.6±6.8 kg/m$^2$, n=25), 6-months (mean BMI 35.7±6.3, n=16), and 1-year following (mean BMI 31.6±6.3 kg/m$^2$, n=15) RYGB surgery as part of a study conducted between 2005 and 2009 at the University of Minnesota-Twin Cities, Minnesota. Partial body composition data from this study have been previously described.[36,45] Briefly, women with extreme obesity who were planning to undergo laparoscopic RYGB were recruited from the Weight Loss Management and Surgery Center at the University of Minnesota Health to take part in a longitudinal study of changes in body composition and nutritional status after RYGB. Ethical approval for this study protocol was obtained from the Institutional Review Board and the General Clinical Research Center (GCRC) at the University of Minnesota. Subjects provided written, informed consent before participating.

## 4.4: Methods

### *4.4.1: NHANES 1999-2004 Dataset*

NHANES is a cross-sectional study that assesses the health and diet of the civilian, non-institutionalized, United States (US) population using a multistage clustered

design. NHANES is administered by the National Center for Health Statistics, Centers for Disease Control and Prevention. Certain demographic groups, including older adults, Mexican-Americans, non-Hispanic blacks, and low-income persons, are over-sampled in this dataset. The NHANES 1999-2000, 2001-2002, and 2003-2004 datasets contain whole body DXA scans, which were acquired with a Hologic QDR-4500A fan-beam densitometer (Hologic, Inc., Bedford, Massachusetts). This NHANES DXA dataset has a large amount of missing data due to the way invalid data were handled in the data file.[186,187] Of the 21,230 eligible DXA participants, non-missing data were obtained from 16,973, or 80% of the eligible population. Due to weight and height restrictions, the likelihood of missing DXA data was related to increasing age, BMI, weight, and height.[186,187] Therefore, the subset of non-missing data may not be treated as a random subsample as it may be biased towards the participants with non-missing data.[186,187] NHANES DXA data sets consist of 5 sets of imputed measurements to account for missing measurements in a large subset of the data set. Observations for individuals where the 5 imputed values were not identical (i.e. where imputation was applied) were excluded from the dataset for the purposes of our analysis. Imputation was not applied to pregnant women or to individuals with amputations, thus these individuals were also excluded from the dataset. Total FM as measured by DXA (DXA FM), total lean body mass with (DXA FFM) and without bone (DXA LST) was extracted from the DXA data for comparison with calculated BIS values for FM and FFM.

BIS data were collected with a HYDRA ECW/ICW Bio-Impedance Spectrum Analyzer (Hydra Model 4200, Xitron Technologies, Inc., San Diego, California). The NHANES BIS data set is smaller than the DXA data set as BIS was only conducted on individuals aged 8 to 49 years. Individuals were excluded from BIS measurements if they were pregnant, had any non-finger or non-toe amputations, had artificial joints, pins, plates or metal in their bodies, had a pacemaker or automatic defibrillator, had coronary stents or metal suture material in the heart, or weighed over 300 lbs.[188] Therefore, as with the DXA data, BIS data is biased towards those who were eligible to complete the BIS examination. BIS data including resistance related to ECW ($R_E$) and ICW ($R_I$) estimated from Cole-modeling, were extracted from the dataset. Anthropometric measures for height and weight were also extracted from the NHANES dataset.

*4.4.2: Longitudinal Dataset*

For a longitudinal validation, we separately generated BIS data and Cole model terms $R_I$ and $R_E$ (Hydra Model 4200, Xitron Technologies, Inc., San Diego, California) from women with obesity before (n=25), 6-months following (n=16) and 1-year following (n=15) RYGB surgery. Whole body BIS measurements were obtained at 10 minutes after participants assumed a supine position, using standard wrist-ankle tetrapolar arrangement of electrodes on the hand and the foot on the right side of the body. The distance between electrodes was measured and remained constant for repeated measurements at follow-up visits. We previously reported BIS precision with the Hydra device; inter-day, inter-observer coefficients of variation (CV) for ECW and ICW

measures with electrode repositioning were 1.28% and 1.72%, respectively.[189] Anthropometric measurements were completed following a standard protocol.[45]

Half-body DXA scans were performed on the same instrument (GE Lunar Prodigy; GEMedical Systems, Madison, WI) for each study visit by an experienced technician at the University of Minnesota General Clinical Research Center Body Composition Laboratory. All scans were performed in "thick" mode using software version 8.8; a modified protocol for conducting half body scans was utilized, as described previously.[45] The precision of the half-body protocol was CV of 5% or less based upon 3 repeat scans with repositioning at baseline.[45]

## 4.5: Calculations

### *4.5.1: Calculation of Xitron-Hanai-Based Mixture Equation Estimated ECW and ICW*

Using the raw frequency data available from the NHANES and longitudinal data sets, we calculated $ECW_{Hydra}$ and $ICW_{Hydra}$ estimates following the standard Xitron-Hanai-based mixture equations as follows, and designated as the Hydra method, given that these are the same equations and constants utilized by the Hydra device software:[31,33]

**Equation 4-1: Xitron-Hanai-based mixture equation for ECW**

$$ECW_{Hydra} = \frac{1}{100}\left(\frac{\rho_{ECW} \cdot K_B \cdot Height^2 \cdot \sqrt{Weight}}{\sqrt{D} \cdot R_0}\right)^{\frac{2}{3}} \quad (1)$$

where $\rho_{ECW}$ is the extracellular resistivity (female: 39 Ωcm, male: 42 Ωcm)[31], $D$ is the body density (defined as 1.05 kg/L), $K_B = 4.3$ and is a factor correcting for body shape in

whole body wrist-ankle measurement, and $R_0$ is resistance at zero frequency which can be considered equivalent to $R_E$.[31,182]

ICW was calculated using the second generation Xitron-Hanai-based mixture equations:[32]

**Equation 4-2: Xitron-Hanai-based mixture equation for ICW**

$$\mathrm{ICW_{Hydra}} = \mathrm{ECW_{Hydra}}\left(\left[\frac{\rho_{TBW}\cdot R_\mathrm{E}+R_\mathrm{I}}{\rho_{ECW}\cdot R_\mathrm{I}}\right]^{\frac{2}{3}}-1\right) \quad (2)$$

where $\rho_{TBW}$ is total body resistivity and is calculated as:

**Equation 4-3: Total body resistivity**

$$\rho_{\mathrm{TBW}} = \rho_{\mathrm{ICW}} - (\rho_{\mathrm{ICW}} - \rho_{\mathrm{ECW}})\cdot\left(\frac{R_\mathrm{I}}{R_\mathrm{E}+R_\mathrm{I}}\right)^{\frac{2}{3}} \quad (3)$$

where $\rho_{ICW}$ is the intracellular resistivity (female: 264.9 Ωcm, male: 273.9 Ωcm)[31].

### *4.5.2: Calculation of FM and FFM From Xitron-Hanai-Based Mixture Equation Estimated ECW and ICW*

FM was calculated from the $ECW_{Hydra}$ and $ICW_{Hydra}$ values using the following relationship where FFM is estimated using the equation:

**Equation 4-4: FFM calculation from Xitron-Hanai-based mixture equations**

$$\mathrm{FFM_{Hydra}} = (d_{\mathrm{ECW}}\times V_{\mathrm{ECW}}) + (d_{\mathrm{ICW}} + V_{\mathrm{ICW}}) \quad (4)$$

where $d_{ECW}$ and $d_{ICW}$ are the mean densities of the ECW and ICW as defined by Xitron ($d_{ECW}$: 1.106 kg/L and $d_{ICW}$:1.521 kg/L, Xitron-Hydra manual)[31], and $V_{ECW}$ and $V_{ICW}$ are the $ECW_{Hydra}$ and $ICW_{Hydra}$ volumes previous determined by equations (1) and (2). Then,

$FM_{Hydra}$ was calculated by subtracting $FFM_{Hydra}$ from the participants' measured weight[188]:

**Equation 4-5: FM calculation from weight and FFM**

$$FM_{Hydra} = Weight - FFM_{Hydra} \quad (5)$$

*4.5.3: Calculation of Moissl BMI-Corrected ECW and ICW Values*

$ECW_{Moissl}$ and $ICW_{Moissl}$ were calculated using the BMI-corrected Xitron-Hanai-based mixture equations equations developed by Moissl et al[182]:

**Equation 4-6: ECW with Moissl correction**

$$ECW_{Moissl} = k_{ECW}\left(\frac{Height^2 \cdot \sqrt{Weight}}{R_E}\right)^{\frac{2}{3}} \quad (6)$$

**Equation 4-7: ICW with Moissl correction**

$$ICW_{Moissl} = k_{ICW}\left(\frac{Height^2 \cdot \sqrt{Weight}}{R_I}\right)^{\frac{2}{3}} \quad (7)$$

where $k_{ECW}$ *and* $k_{ICW}$ are functions of BMI based on height, weight and resistance, as described by Moissl et al.[182]:

**Equation 4-8: Moissl BMI-based correction equations**

$$k_{ECW} = \frac{a}{BMI} + b, \qquad k_{ICW} = \frac{c}{BMI} + d \quad (8)$$

where *a*, *b*, *c*, and *d* are constants determined by Moissl et al. as follows:[182]

$a = 0.188$, $b = 0.2883$, $c = 5.8758$ and $d = 0.4194$.

The resulting $ECW_{Moissl}$ and $ICW_{Moissl}$ were applied to the equations (4) and (5) to generate Moissl-BMI adjusted FM and FFM ($FM_{Moissl}$ and $FFM_{Moissl}$, respectively).

*4.5.4: Generation of Chamney Values for NH_LT, NH_AT and FM From Moissl ECW and ICW*

The $ECW_{Moissl}$ and $ICW_{Moissl}$ values were converted to mass using a value of 0.993 kg/L at 37°C and were applied to the Chamney model[183] to generate values for $ExF_{Moissl}$, $NH\_LT_{Moissl}$, $NH\_AT_{Moissl}$ and $FM_{M\text{-}C}$ using the following equations:[183]

**Equation 4-9: Chamney ExF**

$$ExF_{Moissl} = 1.136 \times ECW_{Moissl} - 0.430 \times ICW_{Moissl} - 0.114 \times Weight \quad (9)$$

**Equation 4-10: Chamney NH_LT**

$$NH\_LT_{Moissl} = 2.725 \times ICW_{Moissl} + 0.191 \times ExF_{Moissl} - 0.191 \times Weight \quad (10)$$

**Equation 4-11: Chamney NH_AT**

$$NH\_AT_{Moissl} = Weight - ExF_{Moissl} - NH\_LT_{Moissl} \quad (11)$$

**Equation 4-12: Chamney FM**

$$FM_{M\text{-}C} = 0.753 \times NH\_AT_{Moissl} \quad (12)$$

It should be noted that the Chamney equations have not been developed to prevent the generation of non-physiological (i.e. ≤0 kg) values of FM, therefore the application of the Chamney equations to the Moissl adjusted values for ECW and ICW resulted in the generation of a small number of observations with negative, non-physiological values for estimated $NH\_AT_{Moissl}$ and $FM_{M\text{-}C}$. For analysis purposes we left these values in the dataset (n=21, mean BMI=19.3±1.5, all male).

*4.5.5: Generation of Chamney Values for NH_LT, NH_AT and FM From Hydra ECW and ICW (i.e. No BMI Correction)*

To evaluate the Chamney model without the correction for BMI in the Xitron-Hanai-based mixture equations, we also applied the Chamney equations (9-12) using the $ECW_{Hydra}$ and $ICW_{Hydra}$ values to generate Chamney values without BMI correction. These values are referred to as $ExF_{Hydra}$, $NH\_LT_{Hydra}$, $NH\_AT_{Hydra}$, and $FM_{H\text{-}C}$.

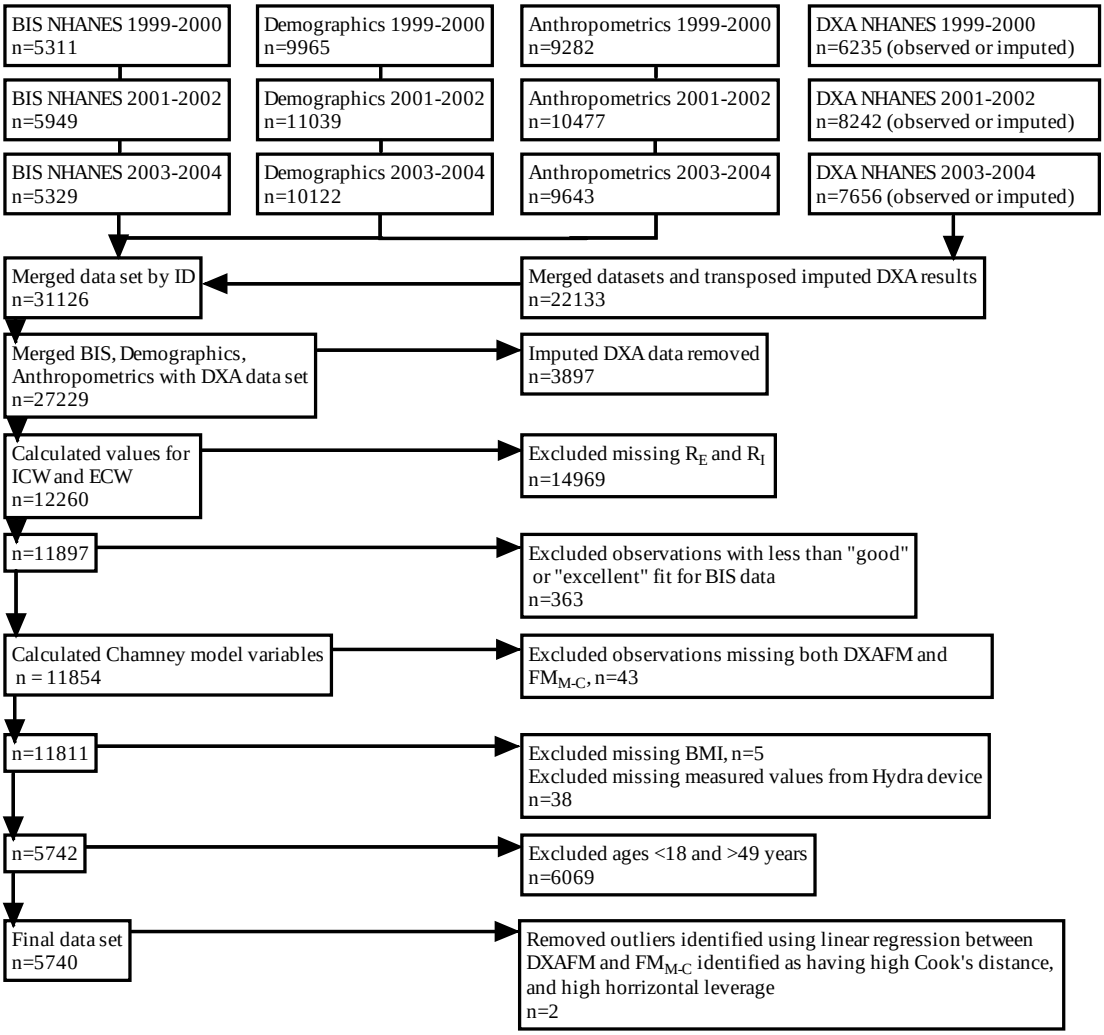
*4.5.6: Selection of Observations for Inclusion in Analysis*

Figure 4-1 is a flowchart that shows how observations were selected for inclusion in the analysis. The BIS, demographic, and anthropometric NHANES datasets for each of the three time periods (1999-2000, 2001-2002, and 2003-2004) were merged by respondent sequence identification (ID) number. The three datasets for the DXA data consist of 5 imputed values for each individual and were transposed before they could be merged with the other data. The DXA data were merged with the BIS, demographic, and anthropometric datasets and observations with imputed values for DXA were removed from the dataset. Weight, height, gender, ethnicity, DXA data, and BIS data were extracted from the datasets. Observations with missing values for $R_E$ and $R_I$ were removed. Calculations for Moissl BMI-adjusted ECW and ICW and unadjusted ECW and ICW were completed, and only BIS data determined to have an 'excellent' or 'good' quality of fit to the Cole model were included in the analysis. Chamney variables were calculated and any observations with missing values for BIS and DXA data or BMI were removed from the dataset. The dataset was limited to observations for individuals between the ages of 18 and 49.

Regression analysis between DXAFM and each of the four methods for FM estimation from BIS data were assessed for outliers. Two outliers (NHANES subject identification numbers 681 and 16964) were identified using regression analysis between DXAFM and $FM_{M\text{-}C}$ and between DXAFM and $FM_{Moissl}$ and determined to have high values for Cook's distance (relative to other observations in the sample) and to also have high horizontal leverage. Observations with high Cook's distance but no horizontal

leverage effect were not removed from the sample. Closer inspection of the two outliers revealed that both had BMI greater than 37 $kg/m^2$, but were otherwise unremarkable. Removal of these two outliers did not significantly alter the results due to the large number of observations include in the analysis. After all exclusions, the resulting dataset consisted of 5740 individuals who had complete information for all variables used for this study.

**Figure 4-1: Flowchart of observation selection from NHANES 1999-2004.**




Abbreviations: BIS, bioimpedance spectroscopy; NHANES, national health and nutrition examination survey; ID, subject identification number; DXA, dual energy X-ray absorptiometry; ICW, intracellular water; ECW, extracellular water; $R_E$, resistance of ECW; $R_I$, resistance of ICW; FM, fat mass; BMI, body mass index; M-C, Moissl-Chamney.

*4.5.7: Statistical analysis*

In both datasets, unless otherwise noted, method agreement for FM by BIS and DXA was reported as mean ± SD, compared between genders, and evaluated for agreement between methods using linear regression analysis, Pearson's correlation (*r*), coefficient of determination ($r^2$), concordance correlation coefficients (CCC), and Bland-Altman (B-A) analysis. B-A analysis included reported values for the bias between the two methods (i.e. the mean difference) as well as the limits of the agreement (1.96 x SD) of the bias. These data allowed for the calculation of percent error (PE) by taking the limits of agreement for the difference between the two methods and dividing by the mean of the reference (DXA) method (referred to as B-A PE). B-A PE <30% was deemed to indicate acceptable agreement between methods. We also discuss error as root mean square error (RMSE) and percent RMSE (%RMSE) whereby %RMSE=RMSE/reference mean x 100. %RMSE <15% was deemed to indicate acceptable error between methods. Where appropriate, the $r^2$ for the regression line for the B-A analysis was reported. Significant $r^2$ between the difference and average between methods, assessed by the B-A plot, indicated a proportional bias. Given the size of the dataset, small deviations from 0 were found to be significant, therefore, only significant $r^2$ values that were also > 0.10 were considered to indicate the presence of a proportional bias. Non-parametric paired t-tests were used for comparison of mean values with P <0.05 used to determine statistical significance. All data analysis was completed using SAS software, versions 9.4 of the SAS System (SAS Institute Inc., Cary, NC, USA).

## 4.6: Results

### *4.6.1: NHANES Dataset Descriptive Statistics*

Descriptive statistics from the NHANES dataset are shown in Table 4-1. From the combined NHANES 1999-2004 datasets body composition data measured by both DXA and BIS was available for 5740 adults between the ages of 18 and 49 years. Of the 5740 individuals, 2744 were female and 2996 were male with an overall mean BMI of 26.7±5.4 kg/m$^2$; 34% identified as Mexican American or Hispanic, 40% identified as non-Hispanic white, 22% identified as non-Hispanic black, and 4% identified as another race or multiracial. Males and females had significantly different mean age, weight, height and BMI. BIS measured values for $R_E$ and $R_I$ differed by gender.

Body composition data for males and females in the NHANES sample is presented in Table 4-2 and includes the measured mean FM, FFM and LST values from DXA as well as the estimated FM and FFM from the Hydra device with and without adjustment for BMI using the Moissl equations. Chamney model values estimated from the BIS raw data, with and without adjustment for BMI using the Moissl equations are also reported. All values except for ExF with and without correction for BMI were different by gender.

**Table 4-1: Descriptive statistics and BIS raw data for the NHANES dataset by gender**

| | All (n=5740) | Male (n=2996) | Female (n=2744) | P-value |
|---|---|---|---|---|
| Age, years | 31.2 ± 10.1 (18-49) | 30.9 ± 10.0 | 31.6 ± 10.2 | 0.007 |
| Weight, kg | 75.9 ± 16.8 (34-138) | 80.5 ± 15.8 | 71.0 ± 16.6 | <0.0001 |
| Height, cm | 168.6 ± 9.7 (133-194) | 174.8 ± 7.6 | 161.9 ± 6.8 | <0.0001 |
| BMI, $kg/m^2$ | 26.7 ± 5.4 (15-52) | 26.3 ± 4.6 | 27.1 ± 6.0 | <0.0001 |
| BIS raw values | | | | |
| $R_E$, Ω | 644.6 ± 96 (406-1075) | 596.8 ± 73.6 | 696.8 ± 90.2 | <0.0001 |
| $R_I$, Ω | 1281 ± 321 (595-2768) | 1088 ± 204 | 1494 ± 291 | <0.0001 |

Abbreviations: BMI, body mass index; BIS, bioimpedance spectroscopy; $R_E$, resistance of extracellular fluid; $R_I$, resistance of intracellular fluid.
Sample values are mean ± SD (range); male and female values are mean ± SD.
P-values are comparison between males and females by student's t-test.
PA is calculated as: $\tan^{-1}(X_{50}/R_{50}) \times (180/\pi)$, and reported in degrees.
IR is calculated as: Z200/Z5, were Z is impedance at 200 kHz and 5 kHz.

**Table 4-2: Mean body composition by method of calculation for the NHANES data set**

| | All | Male | Female | P-value |
|---|---|---|---|---|
| DXA | | | | |
| DXA LST, kg | 49.8 ± 11.2 | 57.5 ± 8.7 | 41.5 ± 8.8 | <0.0001 |
| DXA FFM, kg | 52.2 ± 11.6 | 60.1 ± 9.0 | 43.6 ± 7.1 | <0.0001 |
| DXA FM, kg | 24.4 ± 10.2 | 21.2 ± 8.6 | 28.1 ± 10.7 | <0.0001 |
| % fat by DXA | 31.6 ± 9.1 | 25.5 ± 6.2 | 38.4 ± 6.7 | <0.0001 |
| Hydra | | | | |
| $ECW_{Hydra}$, L | 16.4 ± 3.5 | 18.5 ± 2.9 | 14.0 ± 2.4 | <0.0001 |
| $ICW_{Hydra}$, L | 22.6 ± 6.4 | 26.9 ± 5.2 | 17.9 ± 3.8 | <0.0001 |
| $FFM_{Hydra}$, kg | 52.4 ± 13.3 | 61.3 ± 10.7 | 42.7 ± 8.1 | <0.0001 |
| $FM_{Hydra}$, kg | 23.5 ± 10.7 | 19.2 ± 8.9 | 28.3 ± 10.5 | <0.0001 |
| % fat by Hydra | 30.7 ± 10.8 | 23.2 ± 7.8 | 38.9 ± 7.0 | <0.0001 |
| Moissl-Corrected | | | | |
| $ECW_{Moissl}$, L | 15.9 ± 3.2 | 17.8 ± 2.6 | 13.9 ± 2.3 | <0.0001 |
| $ICW_{Moissl}$, L | 22.4 ± 5.2 | 26.3 ± 3.7 | 18.3 ± 2.7 | <0.0001 |
| $FFM_{Moissl}$, kg | 51.7 ± 11.1 | 59.6 ± 8.4 | 41.1 ± 6.3 | <0.0001 |
| $FM_{Moissl}$, kg | 24.3 ± 12.0 | 20.9 ± 10.8 | 27.9 ± 12.4 | <0.0001 |
| % fat by Moissl | 30.9 ± 11.2 | 24.8 ± 9.2 | 37.6 ± 9.1 | <0.0001 |
| Hydra-Chamney | | | | |
| $ExF_{Hydra,}$ kg | 0.14 ± 1.5 | 0.16 ± 1.6 | 0.12 ± 1.4 | 0.30 |
| $NH\_LT_{Hydra}$, kg | 46.6 ± 15.0 | 57.4 ± 11.6 | 34.8 ± 7.7 | <0.0001 |
| $NH\_AT_{Hydra}$, kg | 29.2 ± 14.4 | 22.9 ± 11.8 | 36.1 ± 13.8 | <0.0001 |
| $FM_{H\text{-}C}$, kg | 22.0 ± 10.8 | 17.3 ± 8.9 | 27.2 ± 10.4 | <0.0001 |
| % fat by Hydra-Chamney | 28.7 ± 11.3 | 20.7 ± 8.2 | 37.3 ± 7.3 | <0.0001 |
| Moissl-Chamney | | | | |
| $ExF_{Moissl}$, kg | -0.3 ± 0.99 | -0.3 ± 1.1 | -0.26 ± 0.9 | 0.003 |
| $NH\_LT_{Moissl}$, kg | 46.2 ± 12.2 | 55.6 ± 8.4 | 35.9 ± 5.6 | <0.0001 |
| $NH\_AT_{Moissl}$, kg | 30.1 ± 16.2 | 25.3 ± 14.4 | 35.4 ± 16.4 | <0.0001 |
| $FM_{M\text{-}C}$, kg | 22.7 ± 12.2 | 19.0 ± 10.8 | 26.7 ± 12.4 | <0.0001 |
| % fat by Moissl-Chamney | 28.8 ± 11.7 | 22.3 ± 9.7 | 35.9 ± 9.5 | <0.0001 |

Abbreviations: DXA, dual energy X-ray absorptiometry; FM, fat mass; FFM, fat free mass; ECW, extracellular water; ICW, intracellular water; ExF, extracellular fluid; NH_LT, non-hydrated lean tissue; NH_AT, non-hydrated adipose tissue.
Values are mean ± SD.
P-values are comparison between males and females by student's t-test.

*4.6.2: Method Agreement for the Assessment of Fat Mass*

We calculated FM using each of the four methods described previously ($FM_{Hydra}$, $FM_{Moissl}$, $FM_{H-C,}$ and $FM_{M-C}$), see Table 4-2. Each method was compared to DXAFM to assess for method agreement. The results of these comparisons are reported in Table 4-3. For the overall data set (n=5740), all four methods systematically underestimated FM (by 0.2-2.5 kg) when compared with DXA ($P<0.0001$). However, all four methods were highly correlated with DXAFM with the $FM_{Moissl}$ and $FM_{M-C}$ methods most highly correlated ($r = 0.96$, $P<0.0001$). The RMSE for $FM_{Hydra}$, $FM_{H-C,}$ $FM_{Moissl}$, and $FM_{M-C}$ were 4.2 (17%), 4.6 (19%), 3.4 (14%), and 3.5 (14%), respectively; indicating that the Moissl correction for BMI decreased the error of the estimates. The application of the Chamney model appeared to slightly increase the error of the estimates. CCC indicated better correlation between DXA and BMI-adjusted methods ($FM_{Moissl} = 0.95$ and $FM_{M-C} = 0.93$) than between DXA and BMI-unadjusted methods ($FM_{Hydra} = 0.92$, $FM_{H-C} = 0.88$). B-A PE was lower for BMI-adjusted methods ($FM_{Moissl} = 29\%$ kg, $FM_{M-C} = 30\%$) than for BMI-unadjusted methods ($FM_{Hydra} = 34\%$, $FM_{H-C} = 37\%$).

**Table 4-3: Methods comparison with DXA for FM generated using Hydra, Moissl, Hydra-Chamney and Moissl-Chamney methods, by BMI category**

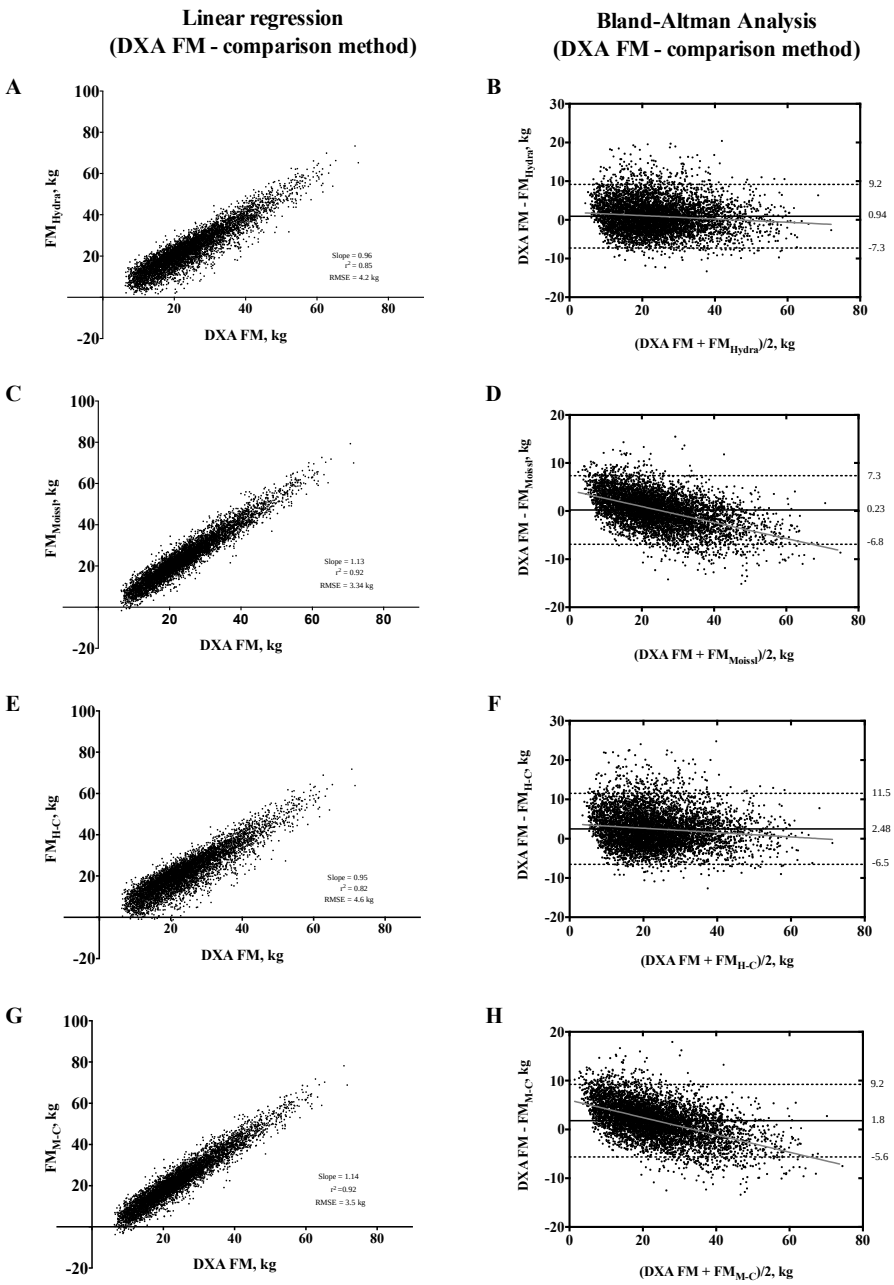
| Comparison | Mean difference, kg (P-value)[a] | $r$ [b] | RMSE, kg | CCC | B-A limits of agreement | $FM_{Method}$ Mean, kg | DXAFM Mean, kg | B-A PE[c] | $r^2$ for regression line through B-A plot (P-value)[d] |
|---|---|---|---|---|---|---|---|---|---|
| **$DXAFM-FM_{Hydra}$** | 0.94 ± 4.2* | 0.92 | 4.2 | 0.916 | -7.3 to 9.2 | 23.5 ± 10.7 | 24.5 ± 10.3 | 34% | 0.01 (<0.0001) |
| <18.5 kg/m$^2$ | -2.2 ± 2.7* | 0.72 | 2.7 | 0.54 | -7.6 to 3.1 | 13.0 ±3.9 | 10.8 ± 2.6 | 49% | 0.30 (<0.0001) |
| 18.5-24.9 kg/m$^2$ | 0.06 ± 3.6 (0.44) | 0.82 | 3.5 | 0.79 | -7.0 to 7.1 | 16.9 ± 6.2 | 16.9 ± 4.7 | 42% | 0.19 (<0.0001) |
| 25-29.9 kg/m$^2$ | 1.5 ± 4.2* | 0.81 | 4.1 | 0.74 | -6.7 to 9.8 | 23.6 ± 7.1 | 25.2 ± 5.1 | 33% | 0.26 (<0.0001) |
| 30-39.9 kg/m$^2$ | 1.9 ± 4.7* | 0.86 | 4.7 | 0.81 | -7.3 to 11.2 | 34.5 ± 9.1 | 36.4 ± 7.0 | 25% | 0.21 (<0.0001) |
| ≥40 kg/m$^2$ | 2.1 ± 5.0* | 0.80 | 5.4 | 0.74 | -7.7 to 12.0 | 51.6 ± 8.4 | 53.8 ± 3.3 | 18% | 0.20 (<0.0001) |
| **$DXAFM-FM_{Moissl}$** | 0.2 ± 3.6* | 0.96 | 3.4 | 0.947 | -6.8 to 7.3 | 24.3 ± 12.1 | 24.5 ± 10.3 | 29% | 0.26 (<0.0001) |
| <18.5 kg/m$^2$ | 2.2 ± 2.6* | 0.74 | 2.6 | 0.55 | -2.8 to 7.3 | 8.6 ± 3.8 | 10.8 ± 2.6 | 47% | 0.27 (<0.0001) |
| 18.5-24.9 kg/m$^2$ | 1.5 ± 3.1* | 0.85 | 3.1 | 0.79 | -4.5 to 7.6 | 15.4 ± 5.9 | 16.9 ± 4.7 | 36% | 0.16 (<0.0001) |
| 25-29.9 kg/m$^2$ | 0.05 ± 3.4 (0.53) | 0.84 | 3.4 | 0.82 | -6.6 to 6.7 | 25.1 ± 6.2 | 25.2 ± 5.1 | 27% | 0.12 (<0.0001) |
| 30-39.9 kg/m$^2$ | -1.8 ± 3.7* | 0.89 | 3.7 | 0.86 | -9.2 to 5.5 | 38.3 ± 8.3 | 36.4 ± 7.0 | 20% | 0.13 (<0.0001) |
| ≥40 kg/m$^2$ | -3.3 ± 3.9* | 0.85 | 3.9 | 0.74 | -10.9 to 4.2 | 57.1 ± 7.3 | 53.8 ± 3.3 | 14% | 0.07 (0.0072) |

| Comparison | Mean difference, kg (P-value)[a] | $r$ [b] | RMSE, kg | CCC | B-A limits of agreement | $FM_{Method}$ Mean, kg | DXAFM Mean, kg | B-A PE[c] | $r^2$ for regression line through B-A plot (P-value)[d] |
|---|---|---|---|---|---|---|---|---|---|
| **DXAFM−$FM_{H-C}$** | 2.5 ± 4.6* | 0.91 | 4.6 | 0.879 | -6.5 to 11.5 | 22.0 ± 10.8 | 24.5 ± 10.3 | 37% | 0.02 (<0.0001) |
| <18.5 kg/m$^2$ | -1.3 ± 3.0* | 0.72 | 2.9 | 0.60 | -7.1 to 4.5 | 12.1 ± 4.2 | 10.8 ± 2.6 | 54% | 0.36 (<0.0001) |
| 18.5-24.9 kg/m$^2$ | 1.4 ± 3.9* | 0.80 | 3.9 | 0.74 | -6.3 to 9.1 | 15.5 ± 6.6 | 16.9 ± 4.7 | 45% | 0.25 (<0.0001) |
| 25-29.9 kg/m$^2$ | 3.2 ± 4.6* | 0.80 | 4.5 | 0.66 | -5.8 to 12.2 | 22.0 ± 7.5 | 25.2 ± 5.1 | 36% | 0.31 (<0.0001) |
| 30-39.9 kg/m$^2$ | 3.7 ± 5.2* | 0.85 | 5.7 | 0.73 | -6.4 to 13.9 | 32.7 ± 9.5 | 36.4 ± 7.0 | 28% | 0.25 (<0.0001) |
| ≥40 kg/m$^2$ | 3.9 ± 5.4* | 0.79 | 5.4 | 0.66 | -6.8 to 14.6 | 49.9 ±8.8 | 53.8 ± 3.3 | 20% | 0.24 (<0.0001) |
| **DXAFM−$FM_{M-C}$** | 1.8 ± 3.8* | 0.96 | 3.5 | 0.93 | -5.6 to 9.2 | 22.7 ± 12.2 | 24.5 ± 10.3 | 30% | 0.27 (<0.0001) |
| <18.5 kg/m$^2$ | 3.6 ± 2.8* | 0.74 | 2.8 | 0.43 | -1.8 to 9.0 | 7.2 ± 4.0 | 10.8 ± 2.6 | 51% | 0.33 (<0.0001) |
| 18.5-24.9 kg/m$^2$ | 3.1 ± 3.3* | 0.85 | 3.2 | 0.71 | -3.4 to 9.5 | 13.8 ± 6.2 | 16.9 ± 4.7 | 38% | 0.22 (<0.0001) |
| 25-29.9 kg/m$^2$ | 1.7 ± 3.6* | 0.84 | 3.7 | 0.78 | -5.3 to 8.7 | 23.5 ± 6.5 | 25.2 ± 5.1 | 28% | 0.17 (<0.0001) |
| 30-39.9 kg/m$^2$ | -0.3 ± 3.9 (0.012) | 0.89 | 3.8 | 0.87 | -7.9 to 7.3 | 36.7 ± 8.5 | 36.4 ± 7.0 | 21% | 0.16 (<0.0001) |
| ≥40 kg/m$^2$ | -2.0 ± 4.0* | 0.84 | 4.0 | 0.80 | -9.8 to 5.9 | 55.7 ± 7.5 | 53.8 ± 3.3 | 15% | 0.09 (0.0019) |

For BMI categories: <18.5 kg/m$^2$, n=170; 18.5-24.9 kg/m$^2$, n=2302; 25-30 kg/m$^2$, n=1876; 30-40 kg/m$^2$, n=1289; >40 kg/m$^2$, n=103. a: Mean difference calculated as dual energy X-ray absorbtometry (DXA) – comparison method. P-value reported by paired t-test. * indicates, P-value <0.0001. b: $r$ =Pearson correlation coefficient. All values are significant, P-value <0.0001. c: Bland-Altman (B-A) percent error (PE) calculated as limits of agreement for the difference between the two methods (1.96(SD)) divided by the mean for the reference DXA method x 100%. d: $r^2$ is reported, P-value indicates significant deviation of regression slope from zero. Unless otherwise noted, all values are mean ± SD. Abbreviations: FM, fat mass; RMSE, root mean square error; CCC, concordance correlation coefficient.

A proportional bias was evident in the B-A assessment for each of the four methods. Although significant, $r^2$ for the regression line through the B-A plot (difference v. average) only accounted for 26% and 27% of the variance in the model for the BMI-corrected methods ($FM_{Moissl}$ and $FM_{M\text{-}C}$, respectively), and accounted for even less (1-2%) of the variance in the BMI-uncorrected methods (see Figure 4-2). Incorporation of the Moissl BMI correction to the Xitron-Hanai-based mixture equations improved the method agreement with the reference DXA method, but also imparted a proportional bias where, as the average of the FM ([DXAFM + FM]/2) increased, the difference between the methods decreased. This proportional bias indicated that the methods did not agree over all weight classes. As can be seen in Figure 4-2, for the B-A analysis of each of the four FM method comparisons with DXA, the x-intercept for regression line (i.e. indicating the average of the FM where there was no bias between methods) was 45.6 kg for $FM_{Hyda}$, 25.7 kg for $FM_{Moissl}$, 68.0 kg for $FM_{H\text{-}C}$, and 33.7 kg for $FM_{M\text{-}C}$. With a lower average of the FM estimates (FM average < regression line x-intercept) there was a larger positive difference between the methods and for higher average FM estimates (FM average > regression line x-intercept) there was a larger negative difference between the methods. For the FM estimates, the slope of the regression line through the B-A plot became slightly more negative after application of the Chamney model to the Moissl BMI-adjusted ECW and ICW values.

**Figure 4-2: Comparison between FM estimates and DXA FM by linear regression and Bland-Altman analysis.**

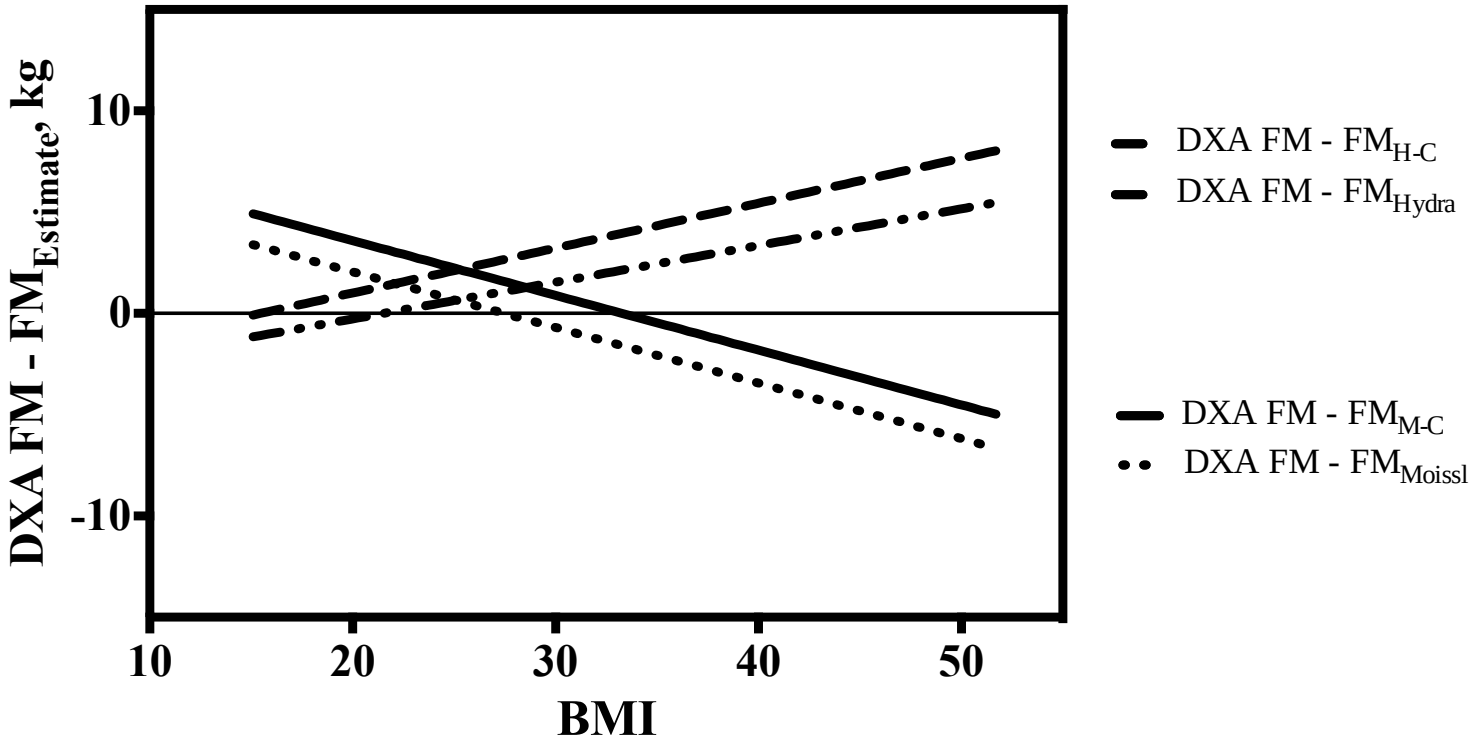



Abbreviations: DXA, dual energy X-ray absorptiometry; FM, fat mass; RMSE, root mean square error; $FM_{Hydra}$, FM calculated from Xitron-Hanai-based mixture equations (A and B); $FM_{Moissl}$, FM corrected for BMI (C and D); $FM_{H-C}$, Chamney FM calculated with ECW and ICW from Hydra (E and F); $FM_{M-C}$, Chameny FM calculated with Moissl-BMI adjusted ECW and ICW (G and H).

*4.6.3: Contribution of BMI to the Error Between Methods*

Using a general linear regression model, we assessed the contribution of age, gender, ethnicity, and BMI on the difference (i.e. the residual) between DXAFM and FM by each of the four BIS methods. We determined that gender, ethnicity and BMI were significant predictors of the difference between DXA and three of the methods tested ($FM_{Hydra}$, $FM_{H-C}$ and $FM_{M-C}$). Both BMI and ethnicity, but not gender, were significant predictors of the difference between DXA and $FM_{Moissl}$. Based on this finding and our interest in the assessment of body composition in individuals with obesity, we completed regression analysis for the difference between DXA FM and FM by each of the four methods (i.e. the residual between methods) with BMI. As can be seen in Figure 4-3 and Table 4-4 comparing the residual for each method to BMI confirmed that BMI was significantly associated with the residual for each method comparison with DXA ($P<0.0001$). However, for Moissl-BMI adjusted methods ($FM_{Moissl}$ and $FM_{M-C}$) this association was negative (slope= -0.27 for both; i.e. larger BMI results in smaller errors), while for non-BMI adjusted methods ($FM_{Hydra}$ and $FM_{H-C}$) this association was positive (slope=0.18 and 0.22 respectively; i.e. larger BMI results in larger errors).

**Figure 4-3: Linear regression analysis for the difference between DXA FM and each of the FM methods across BMI**




Abbreviations: DXA, dual energy X-ray absorptiometry; FM, fat mass; BMI, body mass index; $FM_{Hydra}$, FM calculated from Xitron-Hanai-based mixture equations; $FM_{Moissl}$, FM corrected for BMI; $FM_{H-C}$, Chamney FM calculated with ECW and ICW from Hydra; $FM_{M-C}$, Chamney FM calculated with Moissl-BMI adjusted ECW and ICW.

**Table 4-4: Linear regression analysis for the difference between DXA FM and each of the FM methods across BMI**

| | Model | $r^2$ | RMSE, kg | Intercept | BMI | P-value |
|---|---|---|---|---|---|---|
| DXA FM v. | $FM_{Hydra}$ | 0.05 | 4.08 | -3.89 | 0.18 | <0.0001 |
| | $FM_{Moissl}$ | 0.16 | 3.33 | 7.54 | -0.27 | <0.0001 |
| | $FM_{H-C}$ | 0.06 | 4.46 | -3.42 | 0.22 | <0.0001 |
| | $FM_{M-C}$ | 0.15 | 3.51 | 9.00 | -0.27 | <0.0001 |

Abbreviations: DXA, dual energy X-ray absorptiometry; FM, fat mass; RMSE, root mean square error; BMI, body mass index; $FM_{Hydra}$, FM calculated from Xitron-Hanai-based mixture equations; $FM_{Moissl}$, FM corrected for BMI; $FM_{H-C}$, Chamney FM calculated with ECW and ICW from Hydra; $FM_{M-C}$, Chamney FM calculated with Moissl-BMI adjusted ECW and ICW.

*4.6.4: Assessment of FM Method Agreement by BMI Category*

From the results of our regression analyses and what has been reported in the literature[34,35,182] we hypothesized that BMI category may impact the success of the methods. Table 4-3 shows the assessment of the comparison between DXAFM and each of the four methods by BMI category. BMI categories were defined as underweight (<18.5 kg/m$^2$), normal weight (between 18.5 and 24.9 kg/m$^2$), overweight (between 25 and 29.9 kg/m$^2$), obese (between 30 and 39.9 kg/m$^2$), and severe obesity (≥40 kg/m$^2$).

*4.6.5: Comparison Between DXAFM and $FM_{Hydra}$ by BMI Category*

Below 18.5 kg/m$^2$, the Hydra unadjusted equations systematically overestimated FM relative to DXAFM by ~2.2 kg. Between 18.5 and 24.9 kg/m$^2$, the Hydra equations did not differ from DXAFM. Above 25 kg/m$^2$, the Hydra equations underestimated FM relative to DXAFM by ~1.5 to 2.1 kg. For BMI values <18.5 kg/m$^2$, *r* was less than 0.75, indicating moderate association between methods. For all BMI categories ≥18.5 kg/m$^2$, *r* was >0.8, indicating a stronger association between methods. RMSE ranged from a low value of 2.7 kg for BMI <18.5 kg/m$^2$, and increased with increasing BMI to 5.4 kg for BMI values ≥40 kg/m$^2$, although %RMSE decreased over the same range, from 25% to 10%, respectively. The highest CCC occurred for BMI category 30-39.9 kg/m$^2$ and the lowest occurred for BMI category <18.5 kg/m$^2$, 0.81 and 0.54, respectively. For the BMI range where DXAFM and $FM_{Hydra}$ were not different (18-24.9 kg/m$^2$), CCC was 0.79, indicating moderately strong correlation between methods. B-A PE analysis between methods showed that the highest B-A PE occurred for the <18.5 category, with B-A PE

of 49%; and the lowest occurred for the ≥ 40 kg/m$^2$ category, with B-A PE of 18%. For the BMI range where the methods were not different (18-24.9 kg/m$^2$), with B-A PE of 42%. B-A analysis revealed a proportional bias for all BMI category comparisons.

*4.6.6: Comparison Between DXAFM and $FM_{Moissl}$ by BMI Category*

Below 24.9 kg/m$^2$, the BMI-corrected, Moissl equations systematically underestimated FM relative to DXAFM, by ~1.5 to 2.2 kg. Between 25 and 29.9 kg/m$^2$ the BMI-adjusted FM estimates did not differ from DXAFM. Above 30 kg/m$^2$, Moissl adjusted FM estimates overestimated FM relative to DXAFM by ~1.8 to 3.3 kg. For BMI values <18.5 kg/m$^2$, *r* was less than 0.75, indicating moderate association between methods. For all BMI categories ≥18.5 kg/m$^2$, *r* was>0.85, indicating a strong association between methods. RMSE ranged from a low value of 2.6 kg for BMI <18.5 kg/m$^2$, and increased with increasing BMI to 3.9 kg for BMI values ≥40 kg/m$^2$, although %RMSE decreased from 24% to 7%, respectively over the same range. The highest CCC occurred for BMI category 30-39.9 kg/m$^2$ and the lowest occurred for BMI category <18.5 kg/m$^2$, 0.86 and 0.55, respectively. For the BMI range where DXAFM and $FM_{Moissl}$ were not different (25-29.9 kg/m$^2$), CCC was 0.82, indicating strong correlation between methods. B-A PE analysis between methods showed that the highest PE occurred for the <18.5 kg/m$^2$ category, with B-A PE of 47%; and the lowest occurred for the ≥ 40 kg/m$^2$ category, with B-A PE of 14%. For the BMI range where the methods were not different (25-29.9 kg/m$^2$) B-A PE was 27%. B-A analysis revealed a proportional bias for all BMI category comparisons, except for BMI ≥40 kg/m$^2$. Compared to the BMI-unadjusted

comparison of $FM_{Hydra}$ with DXAFM, the BMI-adjusted comparison between $FM_{Moissl}$ and DXAFM appeared to improve the method for FM estimation.

*4.6.7: Comparison Between DXAFM and $FM_{H-C}$ by BMI Category*

FM estimates using the application of Hydra unadjusted ECW and ICW values to the Chamney equations were different from DXAFM for all BMI categories. Below 18.5 $kg/m^2$, $FM_{H-C}$ systematically overestimated FM relative to DXAFM by ~1.3 kg. Above 18.5 $kg/m^2$, $FM_{H-C}$ underestimated FM relative to DXAFM by ~1.4 to 3.9 kg. For BMI values <18.5, *r* was less than 0.75, indicating a moderate association between methods. For all BMI categories ≥18.5 and <39.9, *r* was>0.80, indicating a stronger association between methods. For BMI values ≥40, *r* was 0.79, indicating a relatively strong association. RMSE ranged from a low value of 2.9 kg for BMI <18.5 $kg/m^2$, and increased with increasing BMI to 5.4 kg for BMI values ≥40 $kg/m^2$, although %RMSE decreased from 27% to 10%, respectively over the same range. The highest CCC occurred for BMI category 18.5-24.9 $kg/m^2$ and the lowest occurred for BMI category <18.5 $kg/m^2$, 0.74 and 0.60, respectively. Compared to other methods, the Hydra-Chamney model was not well correlated with DXAFM. B-A PE analysis between methods showed that the highest B-A PE occurred for the <18.5 category, with B-A PE of 54%; and the lowest occurred for the ≥ 40 $kg/m^2$ category, with B-A PE of 20%. B-A analysis revealed a proportional bias for all BMI category comparisons.

*4.6.8: Comparison Between DXAFM and $FM_{M-C}$ by BMI Category*

Below 29.9 kg/m$^2$, the BMI corrected, Moissl ECW and ICW values applied to the Chamney equations underestimated FM relative to DXAFM. Between 30 and 39.9 kg/m$^2$ the $FM_{M-C}$ estimates did not differ from DXAFM. Above 40 kg/m$^2$, $FM_{M-C}$ estimates overestimated FM relative to DXAFM. For BMI values <18.5 kg/m$^2$, *r* was less than 0.75, indicating moderate association between methods. For all BMI categories ≥18.5 kg/m$^2$, *r* was>0.84, indicating a strong association between methods. RMSE ranged from a low value of 2.8 kg for BMI <18.5 kg/m$^2$, and increased with increasing BMI to 4.0 kg for BMI values ≥40 kg/m$^2$, although %RMSE decreased from 26% to 7%, respectively over the same range. The highest CCC occurred for BMI category 30-39.9 kg/m$^2$ and the lowest occurred for BMI category <18.5 kg/m$^2$, 0.87 and 0.43, respectively. For the BMI range where DXAFM and $FM_{M-C}$ were not different (30-39.9 kg/m$^2$), CCC was 0.87, indicating strong correlation between methods. B-A PE analysis between methods showed that the highest B-A PE occurred for the <18.5 category, with B-A PE of 51%; and the lowest occurred for the ≥ 40 kg/m$^2$ category, with B-A PE of 15%. For the BMI range where the methods were not different (30-39.9 kg/m$^2$), with B-A PE of 21%. B-A analysis revealed a proportional bias for all BMI category comparisons, except for BMI ≥40 kg/m$^2$. Compared to the BMI-unadjusted, Chamney model comparison of $FM_{H-C}$ with DXAFM, the BMI-adjusted, Chamney model comparison between $FM_{M-C}$ and DXAFM appeared to improve the method for FM estimation, particularly in individuals with obesity (BMI ≥ 30 kg/m$^2$).

*4.6.9: Assessment of Moissl-Chamney Model FM by BMI Category and Gender*

Based on the results of the BMI category assessment, we determined that for individuals with obesity (BMI between 30-39.9 kg/m$^2$) the Moissl-Chamney model appeared to be the most effective for estimation of FM from BIS data. To further investigate the application of the Moissl-Chamney model for assessment of FM and to account for gender in the difference between the models, we compared $FM_{Moissl}$ to DXA FM by gender for each BMI category (data not shown). While the proportional bias introduced by the Moissl-BMI correction persisted throughout the genders and categories, the mean difference between the methods decreased as the BMI category increased. The best method agreement appeared to be for men and women with obesity; B-A PE for men with BMI between 30-39.9 kg/m$^2$ was 26% (n = 580, mean difference = -0.37 ± 4.3, P=0.04, *r* = 0.85, mean DXAFM =32.9 kg/m$^2$) and for women with BMI between 30-39 kg/m$^2$ was 17% (n = 709, mean difference = -0.19 ± 3.5, P=0.15, *r* = 0.9, mean DXAFM =39.3 kg/m$^2$). Of note, there was no difference between DXAFM and $FM_{M-C}$ estimates for women with BMI between 30-39 kg/m$^2$.

*4.6.10: Assessment of FFM Method Agreement*

In parallel with our assessment of FM by BMI category, we completed an assessment of BIS estimates of FFM (by Hydra and Moissl) compared with DXA FFM, and NH_LT (by Hydra-Chamney and Moissl-Chamney) compared to DXA LST for the overall data set and by BMI category. Table 4-5 shows the assessment of the comparison between DXA FFM and the two BIS methods used to generate FFM and between DXA

LST and the two BIS methods used to generate NH_LT, by BMI category. Unlike FM estimates, the comparisons between NH_LT and DXA LST are limited and thus, should be interpreted with caution because they do not represent exactly the same tissue compartments due to the differences in methodology.

For the overall data, the $FFM_{Hydra}$ slightly overestimated FFM compared to DXA FFM (by ~0.2 kg). The two methods were well correlated, ($r$ = 0.95, CCC = 0.94) and the error between the methods was relatively low (RMSE = 4.0 kg, 8%). B-A analysis revealed moderate limits of agreement (-8.4 to 8.0 kg) and B-A PE was 16%, however there was a proportional bias. $FFM_{Moissl}$ underestimated FFM compared to DXA FFM. The two methods were also well correlated, ($r$ = 0.95, CCC = 0.95) and the error between the methods was low (RMSE = 3.5 kg, 7%). B-A limits of agreement were relatively small (-6.7 to 7.8 kg) and the B-A PE was 14%. Although statistically significant ($r^2$=0.02, P-value <0.0001), based on our cutpoint of $r^2$<0.10, there was essentially no proportional bias, indicating overall good agreement between the methods.

Because the Moissl-Chamney approach is a multi-component model with a more specific representation of the lean tissue compartment than the more global FFM, it was not deemed to be advantageous from a validation point of view to calculate a FFM from the $FM_{M-C}$ (i.e. weight – $FM_{M-C}$ = $FFM_{M-C}$) for comparison with DXA. However, in the interest of completeness, we did calculate $FFM_{M-C}$ and found that it was well-correlated with DXA FFM for the overall dataset ($r$ = 0.94, p<0.001). The next closest DXA lean

tissue compartment to NH_LT would be the DXA LST, although they reflect different components; for example LST is a more global compartment and includes everything in the FFM except bone. As anticipated, $NH\_LT_{Hydra}$ underestimated lean tissue compared with DXA LST (see Table 4-5). For the overall data, the two methods were well correlated ($r$ = 0.89, CCC=0.83), but there was relatively high error between the methods (RMSE=6.8 kg, 14%), which was expected given that the magnitude of the two compartments was quite different. B-A limits of agreement were wide (-11.2 to 17.5 kg) and B-A PE was 28% with a proportional bias present. Correction for BMI by applying the M-C model to generate $NH\_LT_{Moissl}$ also underestimated mean lean tissue mass compared with DXA LST. Although the two methods were well correlated ($r$ = 0.82, CCC=0.78). Error between the methods was relatively high (RMSE=6.9 kg, 14%). B-A limits of agreement were wide (=10.4 to 17.7 kg) and B-A PE was 28% with a slight proportional error. Therefore, for evaluation of lean tissue in the whole data set the BMI-corrected Moissl equations appeared to have the best agreement with DXA LST. No further analyses on the comparison between DXA LST and $NH\_LT_{Moissl}$ were conducted.

**Table 4-5: Methods comparison for DXA LST and FFM with FFM by Hydra and Moissl, and NH_LT by Hydra-Chamney and Moissl-Chamney methods, by BMI category**

| Comparison | Mean difference, kg (P-value)[a] | $r$[b] | RMSE, kg | CCC | B-A limits of agreement | FFM or $NH\_LT_{Method}$ Mean, kg | DXA Mean, kg | B-A PE[c] | $r^2$ for regression line through B-A plot (P-value)[d] |
|---|---|---|---|---|---|---|---|---|---|
| **DXA FFM− $FFM_{Hydra}$** | -0.20 ± 4.2 (0.0004) | 0.95 | 4.0 | 0.94 | -8.4 to 8.0 | 52.4 ± 13.3 | 52.2 ± 11.6 | 16% | 0.17 (<0.0001) |
| <18.5 kg/m$^2$ | 2.7 ± 2.7* | 0.94 | 2.7 | 0.88 | -2.6 to 8.0 | 37.0 ± 7.8 | 39.7 ± 7.1 | 13% | 0.06 (0.0014) |
| 18.5-24.9 kg/m$^2$ | 0.6 ± 3.6* | 0.95 | 3.5 | 0.94 | -6.5 to 7.7 | 46.9 ± 10.9 | 47.5 ± 9.6 | 15% | 0.14 (<0.0001) |
| 25-29.9 kg/m$^2$ | -0.73 ± 4.2* | 0.94 | 4.1 | 0.93 | -9.0 to 7.5 | 55.1 ± 12.2 | 54.4 ± 10.7 | 15% | 0.13 (<0.0001) |
| 30-39.9 kg/m$^2$ | -1.1 ± 4.7* | 0.94 | 4.6 | 0.93 | -10.4 to 8.2 | 59.8 ± 13.8 | 58.7 ± 12.1 | 16% | 0.14 (<0.0001) |
| ≥40 kg/m$^2$ | -1.6 ± 4.9 (0.001) | 0.87 | 4.9 | 0.83 | -11.3 to 8.1 | 61.0 ± 10.0 | 59.3 ± 7.9 | 16% | 0.19 (<0.0001) |
| **DXA FFM− $FFM_{Moissl}$** | 0.51 ± 3.7* | 0.95 | 3.5 | 0.95 | -6.7 to 7.8 | 51.7 ± 11.1 | 52.2 ± 11.6 | 14% | 0.02 (<0.0001) |
| <18.5 kg/m$^2$ | -1.76 ± 2.6* | 0.95 | 2.6 | 0.92 | -6.8 to 3.3 | 41.4 ± 7.8 | 39.7 ± 7.1 | 13% | 0.07 (0.0003) |
| 18.5-24.9 kg/m$^2$ | -0.88 ± 3.14* | 0.95 | 3.1 | 0.94 | -7.0 to 5.3 | 48.4 ± 10.0 | 47.5 ± 9.6 | 13% | 0.02 (<0.0001) |
| 25-29.9 kg/m$^2$ | 0.77 ± 3.4* | 0.95 | 3.4 | 0.95 | -6.0 to 7.5 | 53.6 ± 10.7 | 54.4 ± 10.7 | 12% | 0.00 (0.85) |
| 30-39.9 kg/m$^2$ | 2.7 ± 3.8* | 0.95 | 3.7 | 0.93 | -4.8 to 10.1 | 56.0 ± 11.6 | 58.7 ± 12.1 | 13% | 0.01 (<0.0001) |
| ≥40 kg/m$^2$ | 3.8±3.8* | 0.89 | 3.7 | 0.79 | -3.6 to 11.2 | 55.5 ± 7.9 | 59.3 ± 7.9 | 13% | 0.00 (0.93) |

| Comparison | Mean difference, kg (P-value)[a] | $r$[b] | RMSE, kg | CCC | B-A limits of agreement | FFM or $NH\_LT_{Method}$ Mean, kg | DXA Mean, kg | B-A PE[c] | $r^2$ for regression line through B-A plot (P-value)[d] |
|---|---|---|---|---|---|---|---|---|---|
| **DXA LST− $NH\_LT_{Hydra}$** | 3.2 ± 7.1* | 0.89 | 6.8 | 0.83 | -11.2 to 17.5 | 46.6 ± 15.0 | 49.8 ± 11.2 | 28% | 0.29 (<0.0001) |
| <18.5 kg/m$^2$ | 5.5 ±4.6* | 0.87 | 4.5 | 0.68 | -3.7 to 14.7 | 32.1 ± 9.0 | 37.6 ± 7.8 | 24% | 0.24 (<0.0001) |
| 18.5-24.9 kg/m$^2$ | 2.9 ± 6.2* | 0.90 | 5.7 | 0.82 | -9.6 to 15.4 | 42.3 ± 13.0 | 45.2 ± 9.2 | 27% | 0.39 (<0.0001) |
| 25-29.9 kg/m$^2$ | 2.3 ± 7.2* | 0.89 | 6.8 | 0.82 | -12.3 to 16.7 | 49.6 ± 14.5 | 51.9 ± 10.3 | 27% | 0.36 (<0.0001) |
| 30-39.9 kg/m$^2$ | 4.3 ± 8.3* | 0.88 | 7.8 | 0.79 | -12.3 to 21.0 | 51.8 ± 16.5 | 56.1 ± 11.7 | 29% | 0.36 (<0.0001) |
| ≥40 kg/m$^2$ | 8.2 ± 8.5* | 0.78 | 8.3 | 0.52 | -8.7 to 25.2 | 48.6 ± 12.8 | 56.8 ± 7.7 | 29% | 0.40 (<0.0001) |
| **DXA LST− $NH\_LT_{Moissl}$** | 3.7 ± 7.0* | 0.82 | 6.9 | 0.78 | -10.4 to 17.7 | 46.2 ± 12.2 | 49.8 ± 11.2 | 28% | 0.02 (<0.0001) |
| <18.5 kg/m$^2$ | -2.5 ± 4.3* | 0.90 | 4.0 | 0.82 | -11.0 to 6.1 | 40.1 ± 9.2 | 37.6 ± 7.8 | 22% | 0.31 (<0.0001) |
| 18.5-24.9 kg/m$^2$ | -0.3 ± 5.2 (0.02) | 0.90 | 5.0 | 0.88 | -10.7 to 10.2 | 45.4 ± 11.7 | 45.2 ± 9.2 | 23% | 0.24 (<0.0001) |
| 25-29.9 kg/m$^2$ | 4.0 ± 5.4* | 0.90 | 5.4 | 0.83 | -6.7 to 14.9 | 47.9 ± 12.3 | 51.9 ± 10.3 | 21% | 0.14 (<0.0001) |
| 30-39.9 kg/m$^2$ | 9.1 ± 6.1* | 0.89 | 6.1 | 0.67 | -2.3 to 22.1 | 46.2 ± 13.1 | 56.1 ± 11.7 | 21% | 0.06 (<0.0001) |
| ≥40 kg/m$^2$ | 16.9 ± 5.9* | 0.78 | 5.8 | 0.26 | 5.28 to 28.5 | 39.9 ± 9.3 | 56.8 ± 7.7 | 20% | 0.09 (0.003) |

For BMI categories: <18.5 kg/m$^2$, n=170; 18.5-24.9 kg/m$^2$, n=2302; 25-30 kg/m$^2$, n=1876; 30-40 kg/m$^2$, n=1289; >40 kg/m$^2$, n=103.
a: Mean difference calculated as dual energy X-ray absorptiometry (DXA) – comparison method. P-value reported by paired t-test. * indicates, P-value <0.0001. b: $r$ = Pearson correlation coefficient. All values are significant, P-value <0.0001. c: Bland-Altman (B-A) percent error (PE) calculated as limits of agreement for the difference between the two methods (1.96(SD)) divided by the mean for the reference DXA method x 100%. d: $r^2$ is reported, P-value indicates significant deviation of regression slope from zero.
Unless otherwise noted, all values are mean ± SD.
Abbreviations: FFM, fat free mass; LST, lean soft tissue; NH_LT, non-hydrated lean tissue; CCC, concordance correlation coefficient.

*4.6.11: Comparison Between DXA FFM and $FFM_{Hydra}$ by BMI Category*

Below 24.9 kg/m$^2$, $FFM_{Hydra}$ underestimates DXA FFM by ~0.6 to 2.7 kg. Above 25 kg/m$^2$, the Hydra unadjusted equations overestimated $FFM_{Hydra}$ relative to DXA FFM by ~0.732 1.6 kg. For BMI values <39.9 kg/m$^2$, *r* was at least 0.94, indicating strong association between methods. Above BMI 40 kg/m$^2$, *r* was slightly decreased to 0.87, which still indicates a strong association between methods. RMSE ranged from a low value of 2.7 kg for BMI <18.5 kg/m$^2$, and increased with increasing BMI to 4.9 kg for BMI values ≥40 kg/m$^2$, although %RMSE remained relatively constant between 7% to 8%, over the same range. The highest CCC occurred for BMI category of 18.5-24.9 kg/m$^2$ and the lowest occurred for BMI category ≥40 kg/m$^2$, 0.94 and 0.83, respectively. B-A PE analysis between methods showed that the highest B-A PE occurred for both the 30-39.9 kg/m$^2$ and ≥40 kg/m$^2$ categories, with B-A PE of 16%; the lowest occurred for the ≤ 18.5 kg category, with B-A PE of 13%. B-A analysis revealed a proportional bias for all BMI category comparisons.

*4.6.12: Comparison Between DXA FFM and $FFM_{Moissl}$ by BMI Category*

For the categories below 24.9 kg/m$^2$, the BMI-corrected Moissl equations overestimated $FFM_{Moissl}$ relative to DXA FFM by ~0.882 1.76 kg. Above 40 kg/m$^2$, the BMI-corrected Moissl equations underestimated $FFM_{Moissl}$ relative to DXA FFM by ~0.772 3.8 kg. For all BMI categories <39.9 kg/m$^2$, *r* was 0.95 and CCC ranged from 0.92-0.95, indicating strong association between methods. Above BMI 40 kg/m$^2$, *r* was

slightly decreased to 0.89, which still indicated a strong association between methods however, CCC=0.79 indicating a moderately strong association. RMSE ranged from a low value of 2.6 kg for BMI <18.5 kg/m$^2$, and increased with increasing BMI to 3.7 kg for BMI values ≥30 kg/m$^2$, although %RMSE remained relatively constant around 6%, over the same range. The highest CCC occurred for BMI category 25-29.9 kg/m$^2$ and the lowest occurred for BMI category ≥40 kg/m$^2$, 0.95 and 0.79, respectively. B-A PE analysis between methods showed that for all BMI categories, B-A PE was 12-13%. B-A analysis revealed no proportional bias for all BMI category comparisons based on our $r^2$ cutpoint of 0.10, and particularly strong evidence for no bias was observed for BMI categories 25-29.9 and ≥40 kg/m$^2$ where the slope was zero and was not statistically significant. Overall, the Moissl FFM equations appear to have moderately good agreement with DXA FFM.

*4.6.13: Longitudinal Dataset and Descriptive Statistics*

After demonstrating that the application of the Moissl-Chamney model was particularly effective for the assessment of FM in overweight women we then applied the model to a group of women with obesity undergoing massive weight loss in the first year after RYGB. Demographic data for the longitudinal dataset including the 6-month and 1-year post surgery time points are shown in Table 4-6. All women in the cohort identified as non-Hispanic white. Body weight and BMI in this cohort were lower at 6-months compared to baseline, and lower at 1-year compared with baseline and 6-months post-

**Table 4-6: Descriptive statistics and BIS raw data, calculated values for BMI adjusted ECW and ICW, BMI adjusted 3-compartment values, and LST and FM from DXA of women undergoing weight loss after RYGB.**

| | Time of follow-up post-RYGB | | |
|---|---|---|---|
| | Baseline (n=25) | 6-months (n=16) | 1-year (n=15) |
| Age at time of surgery, years | 48 ± 10 | 47 ± 10 | 47 ± 10 |
| Weight, kg | 127.4 ± 22.4 | 98.99 ± 19.9[a] | 87.7 ± 19.8[b] |
| Height, cm | 165 ± 6 | 166 ± 5 | 166 ± 5 |
| BMI, $kg/m^2$ | 46.6 ± 6.8 | 35.7 ± 6.3[a] | 31.6 ± 6.3[b] |
| BIS | | | |
| $R_E$, Ω | 545 ± 61 | 586 ± 56 | 582 ± 56 |
| $R_I$, Ω | 1273 ± 138 | 1602 ± 224[a] | 1618 ± 161[a] |
| Moissl | | | |
| $ECW_{Moissl}$, L | 19.9 ± 3.1 | 17.8 ± 2.7[a] | 17.2 ± 2.6[b] |
| $ICW_{Moissl}$, L | 21.4 ± 2.6 | 18.3 ± 2.3[a] | 18.0 ± 1.9[b] |
| Chamney-Moissl | | | |
| $ExF_{Moissl}$, kg | -1.2 ± 1.3 | 1.0 ± 1.0[a] | 1.8 ± 1.1[b] |
| $NH\_LT_{Moissl}$, kg | 33.3 ± 5.5[a] | 30.7 ± 5.6[b] | 32.3 ± 4.9[ab] |
| $NH\_AT_{Moissl}$, kg | 95.3 ± 22.9 | 67.3 ± 21.7[a] | 53.6 ± 21.8[b] |
| $FM_{M-C}$ | 71.8 ± 17.2 | 50.6 ± 16.3[a] | 40.3 ± 16.4[b] |
| DXA | | | |
| DXA FFM | 65.3 ± 12.1 | 55.7 ± 8.4[a] | 56.0 ± 7.3[a] |
| DXA LST | 62.7 ± 12.2 | 52.7 ± 8.3[a] | 53.2 ± 7.1[a] |
| DXA FM | 64.1 ± 13.8 | 44.3 ± 13.6[a] | 33.2 ± 13.3[b] |

Abbreviations: BMI, body mass index; BIS, bioimpedance spectroscopy; $R_E$, resistance of extracellular water; $R_I$, resistance of intracellular water; ECW, extra cellular water; ICW, intracellular water; ExF, excess fluid; NH_LT, non-hydrated lean tissue; NH_AT, non-hydrated adipose tissue; FM, fat mass; DXA, dual energy X-ray absorptiometry; LST, lean soft tissue; RYGB, Roux-en-Y gastric bypass.

[a,b] Means that do not share a letter within a row are significantly different ($P<0.05$) by paired t-test; comparison between baseline and 6-months, n=16; between baseline and 1-year, n=15; between 6-months and 1-year, n=14; means that share a letter within a row are not significantly different by paired t-test.
All values are mean ± SD.

RYGB (P<0.001). BIS values for $R_E$ were not different between visits, however, $R_I$ values were higher than baseline at 6-months and 1-year (P<0.001).

*4.6.14: Comparison Between DXA FM and $FM_{M-C}$ in Obese Women Undergoing Massive Weight Loss*

Analysis of $FM_{M-C}$ compared to DXA FM at the three time points of interest (baseline, 6-months, and 1-year) is displayed in Table 4-7 and shows that the Moissl-Chamney model systematically overestimated FM at each time point. This agreed with the NHANES dataset, where for women with BMI between 30 and 39.9 kg/m$^2$ there was no difference between the two methods, (n=709, DXA FM=39.3 kg v. $FM_{M-C}$=39.5, P=0.15), and for women with BMI ≥ 40 kg/m$^2$ the Moissl-Chamney model overestimated FM compared to DXA (n=89, DXA FM=54.6 v. $FM_{M-C}$ =56.5, P<0.0001). The methods were highly correlated with good CCC values at all time-points. B-A PE was below 30% for each time point (20%, 26%, and 26%) with %RMSE below 15% for each time point (10%, 13%, and 10%). however, the B-A limits of agreement were wide and there was a significant proportional bias for each time point.

As is shown in Table 4-7, when changes in FM by DXAFM and $FM_{M-C}$ between visits were compared using the longitudinal data set, there was no significant difference between the two methods for the weight change intervals from baseline to six months (P=0.3), from baseline to 1-year (P=0.8), and from 6-months to 1-year (P=0.1). Correlation between the two methods was moderate for the change from baseline to 6-

**Table 4-7: Total FM as measured by DXA and as calculated from BIS estimates using the Moissl-Chamney equations for the longitudinal data set.**

| Timepoint | n | Mean difference, kg | *r* | RMSE, kg | CCC | $r^2$ | B-A Limits of agreement | $FM_{M-C}$, kg | DXAFM, kg | B-A PE | $r^2$ for the regression line through the B-A plot (P-value) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-RYGB | 25 | -7.6 ± 6.7* | 0.93* | 6.47 | 0.81 | 0.86 | -20.8 to 5.5 | 71.8 ± 17.2 | 64.1 ± 13.8 | 20% | 0.27 (0.008) |
| 6-months post-RYGB | 16 | -6.3 ± 5.9* | 0.94* | 5.80 | 0.85 | 0.88 | -17.8 to 5.2 | 50.6 ± 16.3 | 44.3 ± 13.6 | 26% | 0.22 (0.07) |
| 1-year post-RYGB | 15 | -7.2 ± 4.3* | 0.98* | 3.45 | 0.86 | 0.96 | -15.7 to 1.3 | 40.3 ± 16.4 | 33.2 ± 13.3 | 26% | 0.53 ( 0.002) |
| Δ Baseline to 6-months | 16 | 1.3 ± 5.3 | 0.68* | 4.26 | 0.64 | 0.46 | -9.1 to 11.8 | -26.5 ± 5.6 | -25.2 ± 7.2 | - | 0.11 ( 0.22) |
| Δ Baseline to 1-year | 15 | 0.36 ± 5.1 | 0.84* | 4.99 | 0.84 | 0.71 | -9.6 to 10.3 | -35.6 ± 8.9 | -35.2 ± 9.2 | - | 0.002 (0.87) |
| Δ 6-months to 1-year | 14 | -1.3 ± 2.8 | 0.88* | 2.83 | 0.86 | 0.78 | -6.9 to 4.2 | -9.2 ± 5.7 | -10.6 ± 5.9 | - | 0.002 (0.87) |

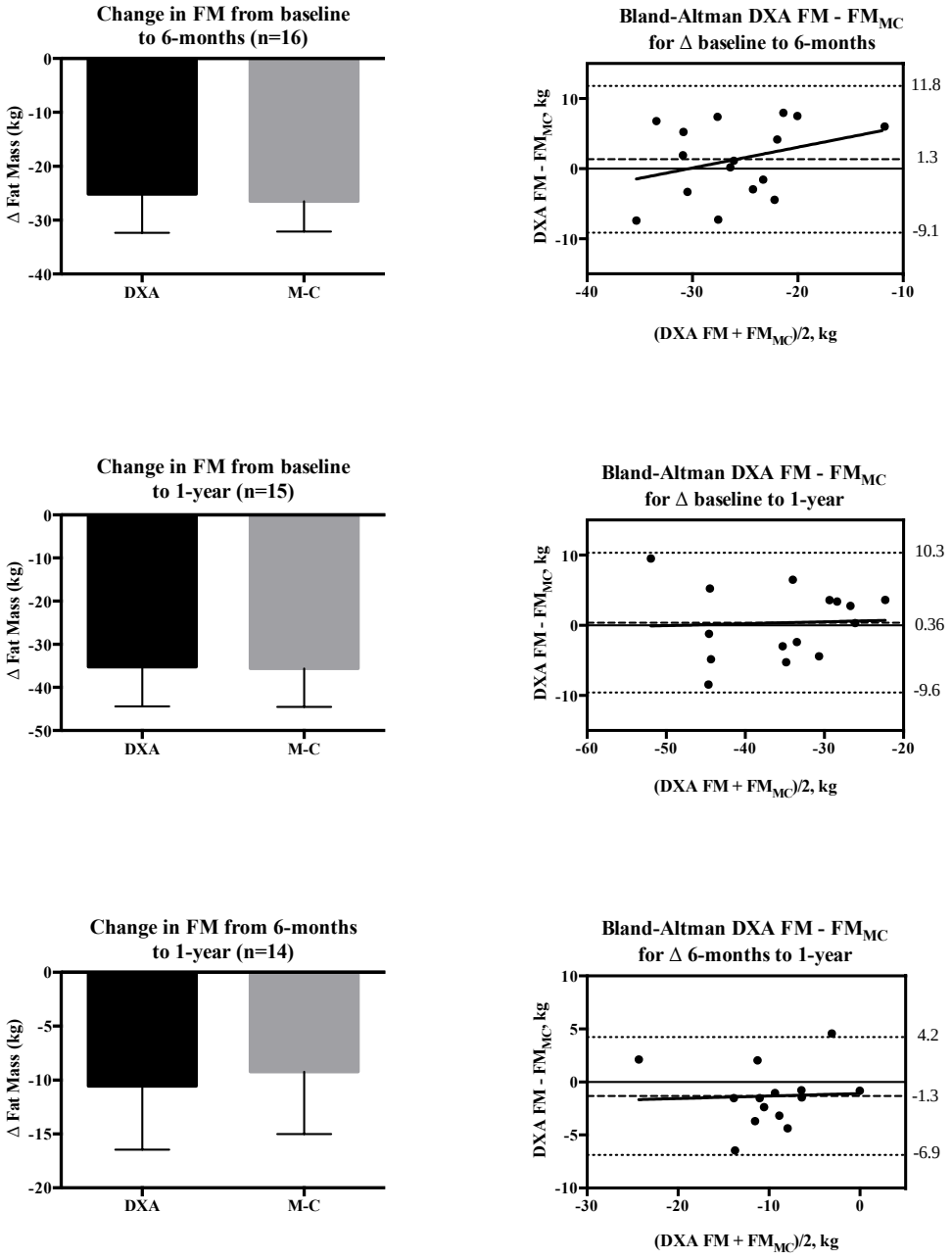
Abbreviations: BIS, bioimpedance spectroscopy; RYGB, Roux-en-Y gastric bypass; DXA, dual energy X-ray absorptiometry; M-C, Moissl-Chamney model; RMSE, root mean square error; B-A, Bland-Altman analysis; $FM_{M-C}$, fat mass from Chamney equation with Moissl correction for BMI; CCC, concordance correlation coefficient; FM, fat mass; PE, percent error. P-E not calculated for FM change intervals because the magnitude of error in all cases was greater than the mean difference between methods.
Values are reported as mean ± SD unless otherwise noted.
* indicates significantly different, P-value <0.001.

months ($r$ = 0.68, P=0.004; CCC = 0.64, 95% CI = 0.23 – 0.86), and well correlated for the change from baseline to 1-year ($r$ = 0.84, P < 0.0001; CCC = 0.84, 95% CI = 0.56 – 0.95) and from 6-months to 1-year ($r$ = 0.88, P < 0.0001; CCC = 0.86, 95% CI = 0.59 – 0.96).

### *4.6.15: B-A Analysis of Change Intervals for DXA FM and $FM_{M-C}$ in Obese Women Undergoing Massive Weight Loss*

B-A analysis of the three change intervals are shown in Figure 4-4. For the change from baseline to six months, bias between the two methods was 1.33 kg with wide limits of agreement of ± 5.33 kg (11.79 kg to -9.12 kg). For the change from baseline to 1-year, bias between the two methods was 0.36 kg with wide limits of agreement of ± 5.08 kg (10.31 kg to -9.6 kg). For the change from 6-months to 1-year, bias between the two methods was -1.32 kg with smaller limits of agreement of ± 2.84 kg (4.24 kg to -6.88 kg). For each of the three change intervals, there was no proportional bias in the B-A plot.

**Figure 4-4: Comparison of DXA FM and Moissl-Chamney FM for weight loss change periods in the first year after RYGB.**




Abbreviations: DXA, dual energy X-ray absorptiometry; FM, fat mass; $FM_{MC}$, fat mass calculated from the Chamney model with BMI-correction from the Moissl equations; M-C, Moissl-Chamney.

*4.6.16: Comparison Between DXA FFM And $FFM_{M-C}$ in Obese Women Undergoing Massive Weight Loss*

The strong method agreement between FM estimates from DXA and the Moissl-Chamney model suggested that it may be worthwhile to also assess the agreement between DXA FFM and $FFM_{M-C}$ in this longitudinal data set. DXA FFM and $FFM_{M-C}$ were significantly correlated at each of the time points [i.e. baseline ($r$ = 0.71, P<0.0001), 6-months ($r$ = 0.85, P<0.0001) and 1-year post-RYGB ($r$ = 0.89, P<0.0001)]. For the change interval from baseline to one-year, the measures of change in DXA FFM and $FFM_{M-C}$ were not different (-11.5 ± 9.8 vs -9.7 ± 2.5 kg, respectively; P=0.48), but were not correlated ($r$ = 0.3, NS). For the change interval from 6-months to one-year post-RYGB, the measures of change in DXA FFM and $FFM_{M-C}$ were different (-0.06 ± 2.8 vs -1.7 ± 2.2 kg, respectively; P=0.03), but were correlated ($r$ = 0.55, P=0.04). In this group, there was an approximately 50% difference of magnitude between $NH\_LT_{Moissl}$ and DXA LST (see Table 4-6); thus we did not make further method comparisons using these lean tissue variables. Interestingly, when evaluating the loss of lean tissue over the one-year follow-up period, our 15 subjects lost an average of 11.5±10.1 kg DXA FFM, 11.6±10.1 kg DXA LST, and 9.4±2.5 kg $FFM_{Moissl}$, , but only 1.3±2.5 kg $NH\text{-}LT_{Moissl}$.

## 4.7: Discussion

We have shown, through use of the NHANES dataset, that BIS can be used to provide reasonably accurate FM and FFM estimates in healthy individuals. We provided further comparison data of BIS methods with DXA through the use of our longitudinal

dataset in women with extreme obesity undergoing RYGB. This study was one of the first to validate a relatively recent BIS approach that incorporates the Moissl[182] BMI-correction to the Xitron-Hanai-based mixture equations and the Chamney 3-component model[183] in a diverse healthy population sample. The strength of the Moissl-Chamney approach is its ability to characterize body composition in terms beyond the 2-compartment (FM and FFM) model. It is particularly compelling from a clinical point of view because it offers the potential to improve BIS estimates of lean tissue by targeting a more specific lean tissue compartment (i.e. NH_LT) than FFM. The importance of lean tissue as it relates to nutritional status is paramount and has gained significant interest in the clinical community as assessment of muscle loss is one of the defining characteristics of malnutrition.[190,191] The use of FFM as a marker of nutritional status is inherently limited, because it is not very specific and includes bone, connective tissue, and extracellular water, among other components. Changes in measured FFM could simply be a reflection of changes in body water (e.g. edema). On the other hand, the NH_LT theoretically represents a compartment closer to what has been termed the body cell mass, reflecting the metabolically active tissue that is thought to be the target of nutritional interventions.[192] Furthermore, an accurate method for quantifying FM changes, particularly in individuals undergoing weight loss interventions would also be of great benefit to clinicians.

In this validation study, our first priority was to compare DXA FM and $FM_{M\text{-}C}$, in part because the direct comparison of NH_LT with LST or FFM by DXA is complicated

by the fact given that there can be a substantial magnitude of difference between them, particularly at higher BMIs. $FM_{M-C}$ is directly calculated from NH_AT, which is derived from NH_LT and ExF, incorporating BMI-corrected ECW and ICW. It is not ideal to calculate $FFM_{M-C}$ by simply subtracting $FM_{M-C}$ from body weight in order to compare with DXA FFM because it increases the potential for error to back-convert to a 2-component model. Thus, we narrowed the focus of our validation effort primarily to the FM measures with a more limited approach to the FFM and NH_LT comparisons. If the agreement between $FM_{M-C}$ and DXA FM were sufficiently strong, then it should follow that the lean estimates could be considered equally valid.

From our analyses of the NHANES dataset (n=5740) inclusive of mixed ethnicity, gender and ages 18-49 years, the Moissl BMI correction of the Xitron-Hanai-based mixture equations ($FM_{Moissl}$) appeared to provide better estimates of FM than uncorrected values ($FM_{Hydra}$) in healthy people. However, a proportional bias was introduced by the BMI-correction that was not previously evident. The Moissl-Chamney approach ($FM_{M-C}$) estimated FM better than $FM_{H-C}$ and equally as well as $FM_{Moissl}$ compared to DXA FM, although the proportional bias was still evident. It appeared that no single BIS approach for FM estimation worked optimally across all BMI ranges in comparison to DXA FM measures. It is important to recognize that BIS techniques were developed from dilution techniques to measure water compartments and thus, using DXA as a reference against which these techniques are compared is somewhat flawed and may be associated with some systematic over- or under-estimation (i.e. scaling error).

Upon closer inspection by BMI-category, it appeared that $FM_{Moissl}$ compared best (no mean bias, lowest B-A PE and %RMSE, reasonably good correlations, and minimal proportional bias) to DXA FM for BMI ≥25 kg/m$^2$. However, $FM_{Moissl}$ systematically overestimated FM at BMI ≥30 kg/m$^2$ and underestimated FM at BMI <25 kg/m$^2$ compared to DXA. Similarly, it appeared that $FM_{M-C}$ performed best at BMI ≥30 kg/m$^2$. However, $FM_{M-C}$ overestimated FM at BMI ≥30 kg/m$^2$ and underestimated at BMI <30 kg/m$^2$. By both methods, proportional bias was quite minimal at BMI ≥40 kg/m$^2$. Taken together, these findings confirm that correcting for BMI may improve FM agreement with DXA for both the Moissl and Moissl-Chamney models at higher BMIs. This is important because the Moissl-Chamney approach, if valid for FM, can potentially provide specific information about the lean tissue compartment as well as hydration status. Further evidence to support this reasoning comes from the comparison of $FFM_{Moissl}$ and DXA FFM, which had high correlation and CCC, low RMSE and low B-A PE with minimal or no proportional bias overall and for all BMI categories in healthy individuals. It is not surprising that the proportional bias disappears when correcting for BMI in the FFM estimates because the equations used to calculate FFM rely heavily on ICW which is adversely affected the most by increasing adiposity.[34]

Our longitudinal study in women undergoing RYGB provided another way to evaluate the Moissl-Chamney approach, in a model of rapid weight loss with substantial body composition changes. These data are uniquely suited for this evaluation given that

mean BMI was ≥30 kg/m$^2$ throughout the study. At all time-points (baseline, 6-months and 1-year post-RYGB) $FM_{M\text{-}C}$ was in good agreement with DXA FM by correlation, %RMSE, and CCC, but systematically overestimated FM compared to DXA FM with B-A PE slightly higher than those reported for the NHANES population, indicating wide individual variability. The comparison of changes were more variable across time, with the strongest support (lowest RMSE, strong correlation and CCC, no proportional bias) for the Moissl-Chamney approach for the change interval between 6-months and 1-year. It should be noted that, as described previously,[45] the quality of the baseline DXA data were suboptimal in some extremely obese individuals which was thought to be due to partial beam attenuation at the trunk causing underestimation of bone estimates. By 6-months, this was no longer an issue due to the substantial weight loss experienced by our subjects. Furthermore, the fact that we conducted half-body scans and used an acrylic board to separate the breast tissue from the arm could have contributed error to the DXA measurement despite correction.[45]

Although the Moissl BMI correction has been validated in healthy individuals and those on dialysis for body water compartments,[182] there are limited published data comparing the Moissl-Chamney approach for FM and lean tissue compartments against reference methods. Two abstracts describing validation efforts for FM and FFM by this approach in healthy individuals and those with cancer, liver, and renal disease are cited[193] but are unavailable in any indexed databases or search engines. Therefore, our ability to compare our results in terms of measurement error against published literature on this

method was limited. In light of this, we have primarily referred to the published literature on body water measurements by the Moissl-BMI correction for comparison purposes.

The interpretation of comparative statistics in methods validation is complicated by the lack of consensus on the definition of acceptable error. Moreover, the level of agreement between methods needed to establish validity for population level studies is different from that needed to apply a method at the individual level for bedside assessment. Although not widely adopted in the body composition field, we utilized B-A PE values with a cut point for method agreement acceptability defined as B-A PE less than 30%. This conception of error has been advocated in the clinical evaluation literature[194–196] and more recently for application to bioimpedance validation studies.[197] More commonly, error is reported as RMSE in absolute terms, and sometimes as % RMSE; however, there is no clear consensus in the field on what constitutes good agreement. We have suggested that %RMSE values <15% represent an acceptable level of error, although we recognize that a low %RMSE does not necessarily ensure good agreement, in that it could be obtained by a method with high precision but high systematic error compared to the reference. From our perspective, we considered evidence of strongest validity to include a combination of correlation >0.9, CCC>0.75, %RMSE <15%, and B-A PE <30%; with no significant mean difference between methods, recognizing that if all other parameters were met, a significant mean difference could signify fixed systematic error (i.e. under- or over-estimation) due to scaling differences. Furthermore, when the errors between methods reflected by the BA analysis

indicated a significant slope, then a systematic proportional error was likely present. Fixed systematic error is potentially less problematic than proportional systematic error, as it could theoretically be corrected with a scalar. The presence of proportional error detracts from a method's validity. Summing this evidence to determine validity is challenging, and whether or not our cut points for error (particularly B-A PE) are sufficiently low to guarantee accuracy at the individual level is unclear. We have attempted to present the most complete picture as possible in our presentation of results.

Much of the BIS literature focuses on the assessment of water volumes using the unadjusted Xitron-Hanai-based mixture equations, rather than FFM or FM, because the BIS method was originally developed for the purpose of measuring ICW and ECW (although these volumes can be used to calculated FFM). There are no published reports comparing FM by the Moissl-Chamney method in any population. Similarly, the very limited BIS Moissl BMI-correction literature that has focused on body composition compartments other than fluid volumes has reported on FFM, rather than FM. Our findings in a healthy diverse US population (18-49 years, n=5740) using the Moissl BMI-correction approach for FFM compared to DXA are similar to those reported by Tengvall et al[198] in an elderly Swedish cohort (75 year old, n=1332). We calculated B-A PE for their $FFM_{Moissl}$ data which was 15% for men and 19% for women; similarly we reported 14% for $FFM_{Moissl}$ in our mixed gender overall dataset. These authors reported no mean difference between $FFM_{Moissl}$ compared to DXA FFM, and the measures were highly correlated ($r$ = 0.93, SEE = 4.4 kg). [198] Because BIS FFM relies on ICW and ECW

estimates and because ICW comprises a major fraction of the FFM, it is useful to look at the BMI-adjusted ICW error estimates (i.e. $ICW_{Moissl}$) in the literature. Based on our interpretation of BA plots from Moissl et al,[182] it appeared that $ICW_{Moissl}$ and $ECW_{Moissl}$ estimates are associated with ~18.5% and ~16.5% B-A PE, respectively, with minimal mean bias, compared to dilution estimates in both healthy individuals (n=120) and those on dialysis (n=32). In our study, we found low error in terms of %RMSE for $FFM_{Moissl}$ (around 6%) compared to DXA FFM; and in earlier work, we reported 6-9 %SEE with no mean bias and high correlations with multiple dilution data in $ICW_{Hydra}$ estimates using the De Lorenzo[31] apparent resistivity constants in individuals with HIV infection.[189] Another widely cited report[199] on the unadjusted BIS approach for ICW and ECW estimates (using older apparent resistivity constants, i.e. not the De Lorenzo constants) was used to calculate B-A PE compared to multiple dilution for healthy individuals indicating 34% and 33% for $ICW_{Hydra}$, and 20% and 28% for $ECW_{Hydra}$ for men and women, respectively. This unadjusted BIS approach also significantly underestimated ICW and overestimated ECW compared to multiple dilution.[199] In a study of individuals with renal disease, we reported $FFM_{Hydra}$ estimates (using the De Lorenzo[31] apparent resistivity constants) that were not different from, and highly correlated with DXA FFM in both healthy individuals (n=23) and those on hemodialysis (n=16) and those not yet on dialysis (n=12).[200] The B-A PE for $FFM_{Hydra}$ estimates were 14%, 20%, and 17%, respectively. In individuals with overweight and obesity (n=90; BMI 23 – 62 $kg/m^2$), Cox-Reijven et al[34] compared $ECW_{Hydra}$ and $TBW_{Hydra}$ using the De Lorenzo[31] apparent resistivity constants to multiple dilution measures, and used the dilution data to generate

new obesity-specific constants. $TBW_{Hydra}$ estimates were not different and highly correlated with dilution, with B-A PE 10 – 11% for the two sets of constants. The $ECW_{Hydra}$ estimates were significantly greater than, but also highly correlated with dilution, and B-A PE were 18% for the two sets of constants. There was a significant proportional bias with increasing BMI that was not corrected by using the obesity-specific resistivity constants, and several of the constants in the equations were also correlated with BMI, underscoring the need to identify other factors that might be corrected in the equations to improve BIS estimates.[34]

Although not an ideal comparison, we found that in healthy individuals $NH\_LT_{Moissl}$ had reasonably good agreement with DXA LST and the Moissl-BMI correction improved these results compared to the agreement between $NH\_LT_{Hydra}$ and DXA LST. As expected, the best agreement was found in normal weight individuals who are unlikely to have issues with excess fluid. In support of this, in our overall NHANES dataset, ExF was quite low (see Table 4-2). This suggests that with normal weight and hydration status DXA LST and NH_LT are measuring very similar lean tissue compartments. At higher BMIs this relationship may not hold because DXA cannot distinguish between overhydrated lean tissue and fluid compartments.

Interestingly, when looking at our longitudinal DXA data (see Table 4-6), it seems that our subjects lost a substantial amount of LST. However, by the BIS Moissl-Chamney approach, the loss of $NH\_LT_{Moissl}$ is fairly minimal. At this point, it would be

difficult to say BIS is providing the correct information, but it is certainly possible that the inability of DXA to distinguish excess fluid in adipose and lean tissue in extreme obesity could be skewing DXA LST loss estimates upward. From a slightly different perspective, this could be interpreted to mean that the observed DXA LST loss was almost entirely adipose tissue water, assuming that the Moissl-Chamney model was valid in these subjects based on our FM change comparisons. This underscores that DXA may be deficient under these conditions and a method such as the BIS Moissl-Chamney approach that can distinguish excess fluid from lean tissue mass could offer significant advantages, particularly for the assessment of clinical populations with edema and individuals with obesity who have abnormal fluid distribution. Indeed, particularly in the most obese state at baseline, our DXA measurements had some error from partial beam attenuation in our heaviest subjects as discussed earlier. On the other hand, the Moissl-Chamney approach can produce negative numbers in the body composition estimates, thus in this case, potentially skewing the NH_LT downward. These issues need to be resolved.

Of note, the Chamney 3-component model was originally developed for the purpose of distinguishing excess fluid from adipose and lean tissue compartments in order to better target the clinical management of individuals on dialysis.[183] Its incorporation in conjunction with the Moissl BMI-correction to the Xitron-Hanai-based mixture equations into software accompanying the BIS device used by Fresenius Medical Care Europe (BCM Body Composition Monitor) has allowed for application of ExF

assessment during dialysis.[184,185,193] Compelling evidence of the efficacy of this approach for lean tissue assessment came from a recent report that demonstrated that individuals on hemodialysis who had NH_LT values below the 10$^{th}$ percentile from healthy reference data had significantly higher mortality, suggesting that it could be indicative of malnutrition and poor clinical status.[185] This approach may ultimately prove to be useful as a way to assess nutritional status and monitor clinical response to nutritional interventions in other populations.

### 4.8: Conclusions

Taken together, the results from our methods comparison between the BIS Moissl-Chamney equations and DXA generally support the utility of this BIS approach for FM, particularly for overweight and obese populations. It also appeared to be a reasonable method for measuring changes in FM with weight loss based on mean level comparisons. However, the presence of proportional bias and wide limits of agreement in the individual time point measures of our longitudinal data set was concerning. It is encouraging that the proportional bias resolved in our measures of FM change, although the apparent systematic overestimation and wide limits of agreement further underscore the need for method refinement before this approach can be relied upon for accurate monitoring of body composition changes at the individual bedside.

We utilized liberal cutpoints to define method acceptability (e.g. B-A PE <30%, %RMSE <15%), assuming the precision of our reference method to be ~5%. Given that

these cutpoints allowed for fairly wide (and probably unacceptable for individual assessment purposes) limits of agreement, it seems likely that a target of B-A PE 10 – 20% and %RMSE of 5 – 10% (or lower) would provide more optimal results, and would allow for the detection of small changes in body composition.

Our findings also emphasize the promise of this technique for the assessment of the more specific lean tissue compartment (NH_LT). Its successful application for assessing excess fluid (ExF) for the tailoring of dialysis regimens provides compelling reason to consider its evaluation in other clinical populations. However, further refinement in the constants used to calculate ExF and the other compartments is needed to protect against the generation of non-physiologic values and to improve the accuracy of the model components. Industry partners that have access to large databases containing both reference (DXA, multiple dilution, 4-component model) and BIS data can work together to further refine Moissl-Chamney types of models to allow for better accuracy and precision in BIS estimates. These kinds of collaborations are essential to move the field forward and resolve questions about the applicability of BIS techniques at the bedside.

Monitoring of lean tissue changes (independent of fluid changes) remains a high priority for nutrition care and monitoring outcomes and success of interventions in the clinical setting of acute and chronic illness. It would be particularly advantageous to have a method to assess lean tissue loss in individuals with obesity, who may not exhibit

visible signs of muscle loss by physical exam, but may be significantly malnourished. Additional research is needed to (1) refine the constants in the BIS Moissl-Chamney model equations using dilution and DXA data from large, diverse population datasets; (2) resolve the issue of non-physiologic values for the BIS Moissl-Chamney model estimates; and (3) confirm if the refined and improved BIS Moissl-Chamney approach or some derivation can be effectively used to monitor body composition changes in individuals with obesity, and in individuals with acute and chronic disease.

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# CHAPTER 5: CONCLUSIONS AND FUTURE DIRECTIONS

Bariatric surgery is highly successful for inducing weight loss and improving metabolic health outcomes. However, the long-term ramifications of bariatric surgery on body composition, and the potential implications of the complex interactions between nutritional status and inflammation on long term health outcomes is not well studied. In part, this is due to the relative recent history of bariatric surgery and the logistical challenges inherent in completing long term follow up studies. Substantial losses of weight and FM in the first year after bariatric surgery are clearly observed. Because of the role that adipose tissue plays in inflammatory processes, these changes could have implications for long term health outcomes.

In our pilot study of 5 women, weight regain occurred and was accompanied by a substantial increase in percent body fat in the long-term post-RYGB. Although we saw no changes in inflammatory and nutritional status at 8.5-years post-RYGB, the finding of weight regain primarily as FM is concerning. In our pilot study, good adherence to recommended supplementation may have offset the risk for nutritional issues. The continued loss of lean tissue with weight regain over time, and the correlation of these losses with loss of muscle strength is also alarming, given its implications for diminished functional status, particularly in light of the aging obese population and the increasing rates of bariatric surgery in the US.

Based on the findings of our pilot study, it seems of vital importance to be able to monitor body composition changes in individuals who undergo bariatric surgery as they age. Available reference methods for the assessment of body composition are costly, cumbersome, and time intensive. Field techniques that are inexpensive, portable, and accurate are needed. Our BIS study evaluated a new application of an existing field technique based on multicomponent physiologic models, and represents a major step forward for measuring changes in body composition after bariatric surgery. With this method we were able to track fat mass and lean tissue changes fairly well in 20 women over the first year post-RYGB. Our findings, in conjunction with a growing literature on the demonstrated effectiveness of this BIS approach in individuals with dialysis for the assessment and monitoring of fluid changes and for the assessment of mortality risk, are compelling. BIS using these models appears to be at the forefront of the bioimpedance field; however, it remains to be seen if additional refinements can lead to its establishment as a useful tool to assess body composition at the individual bedside.

In the coming years, bariatric surgery is sure to remain a popular treatment for obesity and it is clear that we need better methods to assess changes in body composition in a more comprehensive way, in order to better understand the ramifications of these changes in light of long-term nutritionally relevant health outcomes, including inflammation. This dissertation could serve to inform future studies that should aim to tease apart the factors contributing to long-term FM gain, but more importantly loss of LST and muscle strength, to establish evidence based guidelines.

REFERENCES


1. Estimate of Bariatric Surgery Numbers. *Am Soc Metab Bariatr Surg*. 2014.
2. Dimitriadis E, Daskalakis M, Kampa M, Peppe A, Papadakis JA, Melissas J. Alterations in gut hormones after laparoscopic sleeve gastrectomy: a prospective clinical and laboratory investigational study. *Ann Surg*. 2013;257(4):647-654.
3. Barja-Fernández S, Folgueira C, Castelao C, Leis R, Casanueva FF, Seoane LM. Peripheral signals mediate the beneficial effects of gastric surgery in obesity. *Gastroenterol Res Pract*. 2015;2015:560938.
4. Beckman LM, Beckman TR, Sibley SD, et al. Changes in gastrointestinal hormones and leptin after Roux-en-Y Gastric Bypass Surgery. *JPEN*. 2011;35(2):169-180.
5. Lutz TA, Bueter M. The physiology underlying Roux-en-Y gastric bypass: a status report. *Am J Physiol Regul Integr Comp Physiol*. 2014;307(11):R1275-R1291.
6. Buchwald H, Estok R, Fahrbach K, et al. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med*. 2009;122(3):248-256.e5.
7. Stefater MA, Kohli R, Inge TH. Advances in the surgical treatment of morbid obesity. *Mol Aspects Med*. 2013;34(1):84-94.
8. Chouillard EK, Karaa A, Elkhoury M, Greco VJ. Laparoscopic Roux-en-Y gastric bypass versus laparoscopic sleeve gastrectomy for morbid obesity: case-control study. *Surg Obes Relat Dis*. 2011;7(4):500-505.
9. O'Brien PE. Controversies in bariatric surgery. *Br J Surg*. 2015;102(6):611-618.
10. Dixon AFR, Dixon JB, O'Brien PE. Laparoscopic adjustable gastric banding induces prolonged satiety: a randomized blind crossover study. *J Clin Endocrinol Metab*. 2005;90(2):813-819.
11. Burton PR, Brown W, Laurie C, et al. Outcomes, satiety, and adverse upper gastrointestinal symptoms following laparoscopic adjustable gastric banding. *Obes Surg*. 2011;21(5):574-581.
12. Chang S-H, Stoll CRT, Song J, Varela JE, Eagon CJ, Colditz GA. The effectiveness and risks of bariatric surgery: an updated systematic review and meta-analysis, 2003-2012. *JAMA Surg*. 2014;149(3):275-287.
13. Heymsfield SB, Wang Z, Baumgartner RN, Ross R. Human body composition: advances in models and methods. *Annu Rev Nutr*. 1997;17:527-558.
14. Ellis KJ. Human body composition: in vivo methods. *Physiol Rev*. 2000;80(2):649-680.
15. Siri W. Body composition from fluid space and density. In: Brozek J, Hanschel A, eds. *Techniques for Measuring Body Composition*. Vol Washington, DC: National Academy of Science; 1961:223-244.
16. Pietrobelli A, Heymsfield SB, Wang ZM, Gallagher D. Multi-component body composition models: recent advances and future directions. *Eur J Clin Nutr*. 2001;55:69-75.
17. Heymsfield SB, Lichtman S, Baumgartner RN, et al. Body composition of humans: comparison of two improved four-compartment models that differ in

expense, technical complexity, and radiation exposure. *Am J Clin Nutr*. 1990;52:52-58.
18. Duren DL, Sherwood RJ, Czerwinski SA, et al. Body composition methods: comparisons and interpretation. *J Diabetes Sci Technol*. 2008;2(6):1139-1146.
19. Rothney MP, Brychta RJ, Schaefer E V, Chen KY, Skarulis MC. Body composition measured by dual-energy X-ray absorptiometry half-body scans in obese adults. *Obesity (Silver Spring)*. 2009;17(6):1281-1286.
20. Brozek J, Grande F, Anderson JT, Keys A. Densoitometric analysis of body composition: revision of some quantitative assumptions. *Ann N Y Acad Sci*. 1963;110:113-140.
21. Wang Z, Deurenberg P, Wang W, et al. Hydration of fat-free body mass: review and critique of a classic body-composition constant. *Am J Clin Nutr*. 1999;69(5):833-841.
22. Schoeller DA. Hydrometry. In: Heymsfield SB, Lohman TG, Wang Z, Going SG, eds. *Human Body Composition*. Vol 2nd ed. Champaign, IL: Human Kinetics; 2005:35-49.
23. Van Marken Lichtenbelt WD, Kester A, Baarends EM, Westerterp KR. Bromide dilution in adults: optimal equilibration time after oral administration. *J Appl Physiol*. 1996;81(2):653-656.
24. Schlosser K, Kehayias J, Tegenkamp M, Schoeller D. In-vivo and in-vitro bromide equilibration time course in adults and sample handling effect. *Int J Body Compos Res*. 2009;7(4):141-146.
25. Dempster P, Aitkens S. A new air displacement method for the determination of human body composition. *Med Sci Sports Exerc*. 1995;27(12):1692-1697.
26. McCrory MA, Gomez TD, Bernauer EM, Molé PA. Evaluation of a new air displacement plethysmograph for measuring human body composition. *Med Sci Sports Exerc*. 1995;27(12):1686-1691.
27. Sardinha LB, Lohman TG, Teixeira PJ, Guedes DP, Going SB. Comparison of air displacement plethysmography with dual-energy X-ray absorptiometry and 3 field methods for estimating body composition in middle-aged men. *Am J Clin Nutr*. 1998;68(4):786-793.
28. Fuller NJ, Jebb SA, Laskey MA, Coward WA, Elia M. Four-component model for the assessment of body composition in humans: comparison with alternative methods, and evaluation of the density and hydration of fat-free mass. *Clin Sci (Lond)*. 1992;82(6):687-693.
29. Mulasi U, Kuchnia AJ, Cole AJ, Earthman CP. Bioimpedance at the Bedside: Current Applications, Limitations, and Opportunities. *Nutr Clin Pract*. 2015;30(2):180-193.
30. Cole K. *Membranes, Ions and Impulses: A Chapter of Classical Biophysics*. Berkley, CA: University of California Press; 1972.
31. De Lorenzo A, Andreoli A, Matthie J, Withers P. Predicting body cell mass with bioimpedance by using theoretical methods: a technological review. *J Appl Physiol*. 1997;82(5):1542-1558.

32. Matthie JR. Second generation mixture theory equation for estimating intracellular water using bioimpedance spectroscopy. *J Appl Physiol*. 2005;99(2):780-781.
33. Hanai T. Electrical properties of emulsions. In: Sherman PH, ed. *Emulsion Science*. Vol London, UK: Academic Press; 1968:354-377.
34. Cox-Reijven PL, Soeters PB. Validation of bio-impedance spectroscopy: effects of degree of obesity and ways of calculating volumes from measured resistance values. *Int J Obes Relat Metab Disord*. 2000;24(3):271-280.
35. Cox-Reijven PLM, van Kreel B, Soeters PB. Accuracy of bioelectrical impedance spectroscopy in measuring changes in body composition during severe weight loss. *JPEN J Parenter Enteral Nutr*. 2002;26(2):120-127.
36. Mager JR, Sibley SD, Beckman TR, Kellogg T a, Earthman CP. Multifrequency bioelectrical impedance analysis and bioimpedance spectroscopy for monitoring fluid and body cell mass changes after gastric bypass surgery. *Clin Nutr*. 2008;27(6):832-841.
37. Tothill P, Avenell A. Errors in dual-energy X-ray absorptiometry of the lumbar spine owing to fat distribution and soft tissue thickness during weight change. *Br J Radiol*. 1994;67(793):71-75.
38. Waki M, Kral JG, Mazariegos M, Wang J, Pierson RN, Heymsfield SB. Relative expansion of extracellular fluid in obese vs. nonobese women. *Am J Physiol*. 1991;261(2 Pt 1):E199-E203.
39. Das SK, Roberts SB, Kehayias JJ, et al. Body composition assessment in extreme obesity and after massive weight loss induced by gastric bypass surgery. *Am J Physiol Endocrinol Metab*. 2003;284(6):E1080-E1088.
40. Mazariegos M, Kral JG, Wang J, et al. Body composition and surgical treatment of obesity. Effects of weight loss on fluid distribution. *Ann Surg*. 1992;216:69-73.
41. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the United States, 2011-2012. *JAMA*. 2014;311(8):806-814.
42. Mechanick JI, Youdim A, Jones DB, et al. Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient-2013 update: cosponsored by American Association of Clinical Endocrinologists, The Obesity Society, and American Society fo. *Endocr Pract*. 2013;19(2):337-372.
43. Szulc P, Munoz F, Marchand F, Chapurlat R, Delmas PD. Rapid loss of appendicular skeletal muscle mass is associated with higher all-cause mortality in older men: the prospective MINOS study. *Am J Clin Nutr*. 2010;91(5):1227-1236.
44. Park SW, Goodpaster BH, Lee JS, et al. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. *Diabetes Care*. 2009;32(11):1993-1997.
45. Levitt DG, Beckman LM, Mager JR, et al. Comparison of DXA and water measurements of body fat following gastric bypass surgery and a physiological model of body water, fat, and muscle composition. *J Appl Physiol*. 2010;109(3):786-795.
46. Carey DG, Pliego GJ, Raymond RL. Body composition and metabolic changes following bariatric surgery: effects on fat mass, lean mass and basal metabolic rate: six months to one-year follow-up. *Obes Surg*. 2006;16(12):1602-1608.

47. Carrasco F, Papapietro K, Csendes A, et al. Changes in resting energy expenditure and body composition after weight loss following Roux-en-Y gastric bypass. *Obes Surg*. 2007;17(5):608-616.
48. Carrasco F, Ruz M, Rojas P, et al. Changes in bone mineral density, body composition and adiponectin levels in morbidly obese patients after bariatric surgery. *Obes Surg*. 2009;19(1):41-46.
49. Inge T, Wilson K a, Gamm K, Kirk S, Garcia VF, Daniels SR. Preferential loss of central (trunk) adiposity in adolescents and young adults after laparoscopic gastric bypass. *Surg Obes Relat Dis*. 2007;3(2):153-158.
50. Olbers T, Björkman S, Lindroos A, et al. Body composition, dietary intake, and energy expenditure after laparoscopic Roux-en-Y gastric bypass and laparoscopic vertical banded gastroplasty: a randomized clinical trial. *Ann Surg*. 2006;244(5):715-722.
51. Palazuelos-Genis T, Mosti M, Sánchez-Leenheer S, Hernández R, Garduño O, Herrera MF. Weight loss and body composition during the first postoperative year of a laparoscopic Roux-en-Y gastric bypass. *Obes Surg*. 2008;18(1):1-4.
52. Zalesin KC, Franklin B a, Lillystone M a, et al. Differential loss of fat and lean mass in the morbidly obese after bariatric surgery. *Metab Syndr Relat Disord*. 2010;8(1):15-20.
53. Beasley JM, Shikany JM, Thomson C a. The Role of Dietary Protein Intake in the Prevention of Sarcopenia of Aging. *Nutr Clin Pract*. 2013;28(October):684-690.
54. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *J Am Geriatr Soc*. 2002;50:889-896.
55. Faria SL, Faria OP, Buffington C, de Almeida Cardeal M, Ito MK. Dietary protein intake and bariatric surgery patients: a review. *Obes Surg*. 2011;21(11):1798-1805.
56. Bavaresco M, Paganini S, Lima TP, et al. Nutritional course of patients submitted to bariatric surgery. *Obes Surg*. 2010;20(6):716-721.
57. Dobratz JR, Earthman CP. Review of Protein Status and Body Composition After Gastric Bypass Surgery and Very-Low-Calorie Diet Therapy. *Bariatr Nurs Surg Patient Care*. 2006;1(3):195-204.
58. Reid RER, Carver TE, Andersen KM, Court O, Andersen RE. Physical Activity and Sedentary Behavior in Bariatric Patients Long-Term Post-Surgery. *Obes Surg*. 2015:1073-1077.
59. Livhits M, Mercado C, Yermilov I, et al. Behavioral factors associated with successful weight loss after gastric bypass. *Am Surg*. 2010;76(10):1139-1142.
60. Hatoum IJ, Stein HK, Merrifield BF, Kaplan LM. Capacity for physical activity predicts weight loss after Roux-en-Y gastric bypass. *Obesity (Silver Spring)*. 2009;17(1):92-99.
61. Stegen S, Derave W, Calders P, Van Laethem C, Pattyn P. Physical fitness in morbidly obese patients: effect of gastric bypass surgery and exercise training. *Obes Surg*. 2011;21(1):61-70.

62. Castello V, Simões RP, Bassi D, Catai AM, Arena R, Borghi-Silva A. Impact of aerobic exercise training on heart rate variability and functional capacity in obese women after gastric bypass surgery. *Obes Surg*. 2011;21(11):1739-1749.
63. Shah M, Snell PG, Rao S, et al. High-volume exercise program in obese bariatric surgery patients: a randomized, controlled trial. *Obesity (Silver Spring)*. 2011;19(9):1826-1834.
64. Sjöström L, Lindroos A-K, Peltonen M, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med*. 2004;351(26):2683-2693.
65. Courcoulas AP, Christian NJ, Belle SH, et al. Weight change and health outcomes at 3 years after bariatric surgery among individuals with severe obesity. *JAMA*. 2013;310(22):2416-2425.
66. Cooper TC, Simmons EB, Webb K, Burns JL, Kushner RF. Trends in Weight Regain Following Roux-en-Y Gastric Bypass ( RYGB ) Bariatric Surgery. *Obes Surg*. 2015:5-12.
67. Shah M, Simha V, Garg A. Review: long-term impact of bariatric surgery on body weight, comorbidities, and nutritional status. *J Clin Endocrinol Metab*. 2006;91(11):4223-4231.
68. Karmali S, Brar B, Shi X, Sharma AM, de Gara C, Birch DW. Weight recidivism post-bariatric surgery: a systematic review. *Obes Surg*. 2013;23(11):1922-1933.
69. Roslin M, Damani T, Oren J, Andrews R, Yatco E, Shah P. Abnormal glucose tolerance testing following gastric bypass demonstrates reactive hypoglycemia. *Surg Endosc*. 2011;25(6):1926-1932.
70. O'Brien PE, MacDonald L, Anderson M, Brennan L, Brown WA. Long-term outcomes after bariatric surgery: fifteen-year follow-up of adjustable gastric banding and a systematic review of the bariatric surgical literature. *Ann Surg*. 2013;257(1):87-94.
71. Shankar P, Boylan M, Sriram K. Micronutrient deficiencies after bariatric surgery. *Nutrition*. 2010;26(11-12):1031-1037.
72. Alvarez-Leite JI. Nutrient deficiencies secondary to bariatric surgery. *Curr Opin Clin Nutr Metab Care*. 2004;7(5):569-575.
73. Balsa JA, Botella-Carretero JI, Gómez-Martín JM, et al. Copper and zinc serum levels after derivative bariatric surgery: differences between Roux-en-Y Gastric bypass and biliopancreatic diversion. *Obes Surg*. 2011;21(6):744-750.
74. Chen M, Krishnamurthy A, Mohamed AR, Green R. Hematological disorders following gastric bypass surgery: Emerging concepts of the interplay between nutritional deficiency and inflammation. *Biomed Res Int*. 2013;2013.
75. Bakhautdin B, Febbraio M, Goksoy E, et al. Protective role of macrophage-derived ceruloplasmin in inflammatory bowel disease. *Gut*. 2013;62(2):209-219.
76. Bo S, Durazzo M, Gambino R, et al. Associations of dietary and serum copper with inflammation, oxidative stress, and metabolic variables in adults. *J Nutr*. 2008;138(2):305-310.

77. Gletsu-Miller N, Broderius M, Frediani JK, et al. Incidence and prevalence of copper deficiency following roux-en-y gastric bypass surgery. *Int J Obes*. 2012;36(3):328-335.
78. Griffith DP, Liff DA, Ziegler TR, Esper GJ, Winton EF. Acquired copper deficiency: a potentially serious and preventable complication following gastric bypass surgery. *Obesity*. 2009;17(4):827-831.
79. Dalcanale L, Oliveira CPMS, Faintuch J, et al. Long-term nutritional outcome after gastric bypass. *Obes Surg*. 2010;20(2):181-187.
80. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. *Curr Opin Clin Nutr Metab Care*. 2009;12(6):646-652.
81. Prasad AS, Meftah S, Abdallah J, et al. Serum thymulin in human zinc deficiency. *J Clin Invest*. 1988;82(4):1202-1210.
82. Tuerk MJ, Fazel N. Zinc deficiency. *Curr Opin Gastroenterol*. 2009;25(2):136-143.
83. Rojas P, Carrasco F, Codoceo J, et al. Trace element status and inflammation parameters after 6 months of Roux-en-Y gastric bypass. *Obes Surg*. 2011;21:561-568.
84. Beckman LM, Earthman CP, Thomas W, et al. Serum 25(OH) Vitamin D Concentration Changes After Roux-en-Y Gastric Bypass Surgery. *Obesity*. 2013;21(12):E599-E606.
85. Slater G. Serum fat-soluble vitamin deficiency and abnormal calcium metabolism after malabsorptive bariatric surgery. *J Gastrointest Surg*. 2004;8(1):48-55.
86. Zhang Y, Leung DYM, Richers BN, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol*. 2012;188(5):2127-2135.
87. Valerio A, Cardile A, Cozzi V, et al. TNF-alpha downregulates eNOS expression and mitochondrial biogenesis in fat and muscle of obese rodents. *J Clin Invest*. 2006;116(10):2791-2798.
88. Sloka S, Silva C, Wang J, Yong VW. Predominance of Th2 polarization by vitamin D through a STAT6-dependent mechanism. *J Neuroinflammation*. 2011;8:56.
89. Mattner F, Smiroldo S, Galbiati F, et al. Inhibition of Th1 development and treatment of chronic-relapsing experimental allergic encephalomyelitis by a non-hypercalcemic analogue of 1,25-dihydroxyvitamin D(3). *Eur J Immunol*. 2000;30(2):498-508.
90. Kamen DL, Tangpricha V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. *J Mol Med*. 2010;88(5):441-450.
91. Lyakh LA, Sanford M, Chekol S, Young HA, Roberts AB. TGF-beta and vitamin D3 utilize distinct pathways to suppress IL-12 production and modulate rapid differentiation of human monocytes into CD83+ dendritic cells. *J Immunol*. 2005;174(4):2061-2070.
92. Earthman CP, Beckman LM, Masodkar K, Sibley SD. The link between obesity and low circulating 25-hydroxyvitamin D concentrations: considerations and implications. *Int J Obes*. 2012;36(3):387-396.

93. Vimaleswaran KS, Berry DJ, Lu C, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. *PLoS Med*. 2013;10(2):e1001383.
94. Forrest KYZ, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. *Nutr Res*. 2011;31(1):48-54.
95. Gemmel K, Santry HP, Prachand VN, Alverdy JC. Vitamin D deficiency in preoperative bariatric surgery patients. *Surg Obes Relat Dis*. 2009;5(1):54-59.
96. Moizé V, Andreu A, Flores L, et al. Long-term dietary intake and nutritional deficiencies following sleeve gastrectomy or Roux-En-Y gastric bypass in a mediterranean population. *J Acad Nutr Diet*. 2013;113(3):400-410.
97. Damms-Machado A, Friedrich A, Kramer KM, et al. Pre- and postoperative nutritional deficiencies in obese patients undergoing laparoscopic sleeve gastrectomy. *Obes Surg*. 2012;22(6):881-889.
98. Fish E, Beverstein G, Olson D, Reinhardt S, Garren M, Gould J. Vitamin D status of morbidly obese bariatric surgery patients. *J Surg Res*. 2010;164(2):198-202.
99. Coupaye M, Breuil MC, Rivière P, et al. Serum vitamin D increases with weight loss in obese subjects 6 months after Roux-en-Y gastric bypass. *Obes Surg*. 2013;23(4):486-493.
100. Goldner WS, Stoner JA, Thompson J, et al. Prevalence of vitamin D insufficiency and deficiency in morbidly obese patients: a comparison with non-obese controls. *Obes Surg*. 2008;18(2):145-150.
101. Compher CW, Badellino KO, Boullata JI. Vitamin D and the bariatric surgical patient: a review. *Obes Surg*. 2008;18(2):220-224.
102. De Luis DA, Pacheco D, Izaola O, Terroba MC, Cuellar L, Cabezas G. Micronutrient status in morbidly obese women before bariatric surgery. *Surg Obes Relat Dis*. 2013;9(2):323-327.
103. Johnson JM, Maher JW, DeMaria EJ, Downs RW, Wolfe LG, Kellum JM. The long-term effects of gastric bypass on vitamin D metabolism. *Ann Surg*. 2006;243(5):701-704.
104. Khandalavala BN, Hibma PP, Fang X. Prevalence and persistence of vitamin D deficiency in biliopancreatic diversion patients: a retrospective study. *Obes Surg*. 2010;20(7):881-884.
105. Ybarra J, Sánchez-Hernández J, Gich I, et al. Unchanged hypovitaminosis D and secondary hyperparathyroidism in morbid obesity after bariatric surgery. *Obes Surg*. 2005;15(3):330-335.
106. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health. *Am J Clin Nutr*. 2008;87:1080S - 1086S.
107. Scibora LM, Ikramuddin S, Buchwald H, Petit M a. Examining the link between bariatric surgery, bone loss, and osteoporosis: a review of bone density studies. *Obes Surg*. 2012;22(4):654-667.
108. Pramyothin P, Holick MF. Vitamin D supplementation: guidelines and evidence for subclinical deficiency. *Curr Opin Gastroenterol*. 2012;28(2):139-150.

109. Ross AC, Taylor CL, Yaktine AL, Del HB. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: Institute of Medicine of the National Academies; 2011:1-1133.
110. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911-1930.
111. Rosen CJ, Abrams SA, Aloia JF, et al. IOM committee members respond to Endocrine Society vitamin D guideline. *J Clin Endocrinol Metab*. 2012;97(4):1146-1152.
112. O'Brien PE. Bariatric surgery: mechanisms, indications and outcomes. *J Gastroenterol Hepatol*. 2010;25(8):1358-1365.
113. Saltzman E, Karl JP. Nutrient deficiencies after gastric bypass surgery. *Annu Rev Nutr*. 2013;33:183-203.
114. Boullata JI. A rational approach to vitamin D supplementation. *Nutrition*. 2012;28(11-12):1204-1205.
115. Heaney RP, Recker RR, Grote J, Horst RL, Armas LAG. Vitamin D3 is more potent than vitamin D2 in humans. *J Clin Endocrinol Metab*. 2011;96(3):E447-E452.
116. Aarts E, van Groningen L, Horst R, et al. Vitamin D absorption: consequences of gastric bypass surgery. *Eur J Endocrinol*. 2011;164(5):827-832.
117. Heaney RP, Horst RL, Cullen DM, Armas LAG. Vitamin D 3 Distribution and Status in the Body. *J Am Coll Nutr*. 2009;28(3):252-256.
118. Drincic AT, Armas LAG, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity*. 2012;20(7):1444-1448.
119. Duncan A, Talwar D, McMillan DC, Stefanowicz F, O'Reilly DSJ. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. *Am J Clin Nutr*. 2012;95(1):64-71.
120. El-Kadre LJ, Rocha PRS, de Almeida Tinoco AC, Tinoco RC. Calcium metabolism in pre- and postmenopausal morbidly obese women at baseline and after laparoscopic Roux-en-Y gastric bypass. *Obes Surg*. 2004;14(8):1062-1066.
121. DiGiorgi M, Daud A, Inabnet WB, et al. Markers of bone and calcium metabolism following gastric bypass and laparoscopic adjustable gastric banding. *Obes Surg*. 2008;18(9):1144-1148.
122. Sinha N, Shieh A, Stein EM, et al. Increased PTH and 1.25(OH)2D levels associated with increased markers of bone turnover following bariatric surgery. *Obesity*. 2011;19(12):2388-2393.
123. Gehrer S, Kern B, Peters T, Christoffel-Courtin C, Peterli R. Fewer nutrient deficiencies after laparoscopic sleeve gastrectomy (LSG) than after laparoscopic Roux-Y-gastric bypass (LRYGB)-a prospective study. *Obes Surg*. 2010;20(4):447-453.

124. Carlin AM, Rao DS, Yager KM, Parikh NJ, Kapke A. Treatment of vitamin D depletion after Roux-en-Y gastric bypass: a randomized prospective clinical trial. *Surg Obes Relat Dis*. 2009;5(4):444-449.
125. Newbury L, Dolan K, Hatzifotis M, Low N, Fielding G. Calcium and vitamin D depletion and elevated parathyroid hormone following biliopancreatic diversion. *Obes Surg*. 2003;13(6):893-895.
126. Aasheim ET, Johnson LK, Hofsø D, Bøhmer T, Hjelmesæth J. Vitamin status after gastric bypass and lifestyle intervention: a comparative prospective study. *Surg Obes Relat Dis*. 2011;8(2):169-175.
127. Gasteyger C, Suter M, Gaillard RC, Giusti V. Nutritional deficiencies after Roux-en-Y gastric bypass for morbid obesity often cannot be prevented by standard multivitamin supplementation. *Am J Clin Nutr*. 2008;87(5):1128-1133.
128. Flores L, Osaba MJM, Andreu A, Moizé V, Rodríguez L, Vidal J. Calcium and vitamin D supplementation after gastric bypass should be individualized to improve or avoid hyperparathyroidism. *Obes Surg*. 2010;20(6):738-743.
129. Aasheim ET, Björkman S, Søvik TT, et al. Vitamin status after bariatric surgery: a randomized study of gastric bypass and duodenal switch. *Am J Clin Nutr*. 2009;90(1):15-22.
130. Goldner WS, Stoner JA, Lyden E, et al. Finding the optimal dose of vitamin D following Roux-en-Y gastric bypass: a prospective, randomized pilot clinical trial. *Obes Surg*. 2009;19(2):173-179.
131. Mahlay NF, Verka LG, Thomsen K, Merugu S, Salomone M. Vitamin D status before Roux-en-Y and efficacy of prophylactic and therapeutic doses of vitamin D in patients after Roux-en-Y gastric bypass surgery. *Obes Surg*. 2009;19(5):590-594.
132. Stein EM, Strain G, Sinha N, et al. Vitamin D insufficiency prior to bariatric surgery: risk factors and a pilot treatment study. *Clin Endocrinol (Oxf)*. 2009;71(2):176-183.
133. Einarsdóttir K, Preen DB, Clay TD, Kiely L, Holman CDJ, Cohen LD. Effect of a single "megadose" intramuscular vitamin D (600,000 IU) injection on vitamin D concentrations and bone mineral density following biliopancreatic diversion surgery. *Obes Surg*. 2010;20(6):732-737.
134. Moore CE, Sherman V. Vitamin D supplementation efficacy: sleeve gastrectomy versus gastric bypass surgery. *Obes Surg*. April 2014:Epub ahead of print.
135. Su Z, Narla SN, Zhu Y. 25-Hydroxyvitamin D: analysis and clinical application. *Clin Chim Acta*. 2014;433C:200-205.
136. Martins-Costa P, Martins H, Bravo F, Cruz M, Reis J, Oliveira JC. Comparison of automated methods for measurement of 25-hydroxyvitamin D. *Clin Lab*. 2013;59(7-8):885-891.
137. Su Z, Slay BR, Carr R, Zhu Y. The recognition of 25-hydroxyvitamin D2 and D3 by a new binding protein based 25-hydroxyvitamin D assay. *Clin Chim Acta*. 2013;417:62-66.
138. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr*. 2000;72(3):690-693.

139. Arunabh S, Pollack S, Yeh J, Aloia JF. Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab*. 2003;88(1):157-161.
140. Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol (Oxf)*. 2012;76(3):315-325.
141. Li YC. Vitamin D regulation of the renin-angiotensin system. *J Cell Biochem*. 2003;88(2):327-331.
142. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. *Int J Endocrinol*. 2010;2010:351385.
143. Rosen CJ, Adams JS, Bikle DD, et al. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. *Endocr Rev*. 2012;33(3):456-492.
144. Youssef DA, Miller CW, El-Abbassi AM, et al. Antimicrobial implications of vitamin D. *Dermatoendocrinol*. 2011;3(4):220-229.
145. Quraishi SA, Bittner EA, Blum L, Hutter MM, Camargo CA. Association between preoperative 25-hydroxyvitamin D level and hospital-acquired infections following Roux-en-Y gastric bypass surgery. *JAMA Surg*. 2014;149(2):112-118.
146. Fujioka K, DiBaise JK, Martindale RG. Nutrition and metabolic complications after bariatric surgery and their treatment. *JPEN*. 2011;35(5 Suppl):52S - 59S.
147. Pieracci FM, Barie PS, Pomp A. Critical care of the bariatric patient. *Crit Care Med*. 2006;34(6):1796-1804.
148. Visser M, Deeg DJH, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab*. 2003;88(12):5766-5772.
149. Cipriani C, Romagnoli E, Carnevale V, et al. Effect of a single oral dose of 600,000 IU of cholecalciferol on muscle strength: a study in young women. *J Endocrinol Invest*. 2013;36(11):1051-1054.
150. Mete Civelek G, Ozunlu Pekyavas N, Cetin N, Cosar SN, Karatas M. Association of vitamin D deficiency with muscle strength and quality of life in postmenopausal women. *Climacteric*. March 2014:Epub ahead of print.
151. Snijder MB, van Schoor NM, Pluijm SMF, van Dam RM, Visser M, Lips P. Vitamin D status in relation to one-year risk of recurrent falling in older men and women. *J Clin Endocrinol Metab*. 2006;91(8):2980-2985.
152. Sadat-Ali M, Bubshait DA, Al-Turki HA, Al-Dakheel DA, Al-Olayani WS. Topical delivery of vitamin D3: a randomized controlled pilot study. *IJBS*. 2014;10(1):21-24.
153. Blachar A, Federle MP, Pealer KM, Ikramuddin S, Schauer PR. Gastrointestinal Complications of Laparoscopic Roux-en-Y Gastric Bypass Surgery: Clinical and Imaging Findings. *Radiology*. 2002;223(3):625-632.
154. Elliot K. Nutritional considerations after bariatric surgery. *Crit Care Nurs Q*. 2003;26(2):133-138.
155. Pugnale N, Giusti V, Suter M, et al. Bone metabolism and risk of secondary hyperparathyroidism 12 months after gastric banding in obese pre-menopausal women. *Int J Obes Relat Metab Disord*. 2003;27(1):110-116.

156. Faintuch J, Matsuda M, Cruz MELF, et al. Severe protein-calorie malnutrition after bariatric procedures. *Obes Surg*. 2004;14(2):175-181.
157. Del Genio F, Alfonsi L, Marra M, et al. Metabolic and nutritional status changes after 10% weight loss in severely obese patients treated with laparoscopic surgery vs integrated medical treatment. *Obes Surg*. 2007;17(12):1592-1598.
158. Gómez JM, Vilarrasa N, Masdevall C, et al. Regulation of bone mineral density in morbidly obese women: a cross-sectional study in two cohorts before and after bypass surgery. *Obes Surg*. 2009;19(3):345-350.
159. Von Mach M–., Stoeckli R, Bilz S, Kraenzlin M, Langer I, Keller U. Changes in bone mineral content after surgical treatment of morbid obesity. *Metabolism*. 2004;53(7):918-921.
160. Paddon-jones D, Short KR, Campbell WW, Volpi E, Wolfe RR. Role of dietary protein in the sarcopenia of aging 1 – 4. 2008;87:1562-1566.
161. Wolfe RR. The underappreciated role of muscle in health and disease 1 ‡ 3. 2006:475-482.
162. Sergi G, Lupoli L, Busetto L, et al. Changes in Fluid Compartments and Body Composition in Obese Women after Weight Loss Induced by Gastric Banding. *Ann Nutr Metab*. 2003;47(3-4):152-157.
163. Zimmerman ME, Andersson H, Lundell L, Olbe L. Alterations in body composition after gastroplasty for morbid obesity. *Scand J Gastroenterol*. 1990;25(3):263-268.
164. Lohman TG, Roche AF. *Anthropometric Standardization Reference Manual*. Vol (Lohman T, Roche A, Martorell R, eds.). Champaign, IL: Human Kinetics; 1991.
165. Trabulsi J, Troiano RP, Subar AF, et al. Precision of the doubly labeled water method in a large-scale application: evaluation of a streamlined-dosing protocol in the Observing Protein and Energy Nutrition (OPEN) study. *Eur J Clin Nutr*. 2003;57(11):1370-1377.
166. Schoeller DA, van Santen E, Peterson DW, Dietz W, Jaspan J, Klein PD. Total body water measurement in humans with 18O and 2H labeled water. *Am J Clin Nutr*. 1980;33(12):2686-2693.
167. Miller E. Bromide space determination using anion-exchange chromatography for measurement of bromide. *Am J Clin Nutr*. 1989;50(1):168-171.
168. Miller ME, Cappon CJ. Anion-exchange chromatographic determination of bromide in serum. *Clin Chem*. 1984;30(5):781-783.
169. Collins AL, McCarthy HD. Evaluation of factors determining the precision of body composition measurements by air displacement plethysmography. *Eur J Clin Nutr*. 2003;57:770-776.
170. Carver TE, Christou N V., Court O, Lemke H, Andersen RE. In Vivo Precision of the GE Lunar iDXA for the Assessment of Lumbar Spine, Total Hip, Femoral Neck, and Total Body Bone Mineral Density in Severely Obese Patients. *J Clin Densitom*. 2014;17(1):109-115.
171. Huizenga R, Oates MK. Precision of Lunar iDXA total body BMD and composition measurements on obese subjects. *J Clin Densitom*. 2007;10:208.

172. Bosy-Westphal A, Schautz B, Later W, Kehayias JJ, Gallagher D, Müller MJ. What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population. *Eur J Clin Nutr*. 2013;67 Suppl 1:S14-S21.
173. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402-408.
174. Tataranni A, Ravussin E. Use of dual-energy X-ray absorptiometry in obese individuals. *Am J Clin Nutr*. 1995;62(4):730-734.
175. Zhou X, Fragala MS, McElhaney JE, Kuchel GA. Conceptual and methodological issues relevant to cytokine and inflammatory marker measurements in clinical research. *Curr Opin Clin Nutr Metab Care*. 2010;13(5):541-547.
176. Leemasawatdigul K, Gappa-Fahlenkamp H. Effect of storage conditions on the stability of recombinant human MCP-1/CCL2. *Biologicals*. 2011;39(1):29-32.
177. Goktas Z, Moustaid-Moussa N, Shen CL, Boylan M, Mo H, Wang S. Effects of bariatric surgery on adipokine-induced inflammation and insulin resistance. *Front Endocrinol (Lausanne)*. 2013;4(June):1-13.
178. Poggi M, Jager J, Paulmyer-Lacroix O, et al. The inflammatory receptor CD40 is expressed on human adipocytes: Contribution to crosstalk between lymphocytes and adipocytes. *Diabetologia*. 2009;52:1152-1163.
179. Catalán V, Gómez-Ambrosi J, Ramirez B, et al. Proinflammatory cytokines in obesity: impact of type 2 diabetes mellitus and gastric bypass. *Obes Surg*. 2007;17(11):1464-1474.
180. De Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. *BMC Immunol*. 2009;10(1):52.
181. Farrell C-JL, Martin S, McWhinney B, Straub I, Williams P, Herrmann M. State-of-the-art vitamin D assays: a comparison of automated immunoassays with liquid chromatography-tandem mass spectrometry methods. *Clin Chem*. 2012;58(3):531-542.
182. Moissl UM, Wabel P, Chamney PW, et al. Body fluid volume determination via body composition spectroscopy in health and disease. *Physiol Meas*. 2006;27(9):921-933.
183. Chamney PW, Wabel P, Moissl UM, et al. A whole-body model to distinguish excess fluid from the hydration of major body tissues. *Am J Clin Nutr*. 2007;85(1):80-89.
184. Broers NJH, Martens RJH, Cornelis T, et al. Body Composition in Dialysis Patients: A Functional Assessment of Bioimpedance Using Different Prediction Models. *J Ren Nutr*. October 2014:1-8.
185. Marcelli D, Usvyat LA, Kotanko P, et al. Body Composition and Survival in Dialysis Patients: Results from an International Cohort Study. *Clin J Am Soc Nephrol*. April 2015.
186. National Center for Health Statistics. National Health and Nutrition Examination Survey: Technical Documentation for the 1999-2004 Dual Energy X-Ray

Absorptiometry (DXA) Multiple Imputation Data files. *Available at http://www.cdc.gov/nchs/data/nhanes/bc.pdf*. 2008;(February).
187. Schenker N, Borrud LG, Burt VL, et al. Multiple imputation of missing dual-energy X-ray absorptiometry data in the National Health and Nutrition Examination Survey. *Stat Med*. 2011;30(3):260-276.
188. NHANES 2003 - 2004: Bioelectrical Impedance Analysis Data Documentation, Codebook, and Frequencies. *Centers Dis Control Prev*. 2004.
189. Earthman CP, Matthie JR, Reid PM, Harper IT, Ravussin E, Howell WH. A comparison of bioimpedance methods for detection of body cell mass change in HIV infection. *J Appl Physiol*. 2000;88(3):944-956.
190. White J V, Guenter P, Jensen G, Malone A, Schofield M. Consensus statement of the Academy of Nutrition and Dietetics/American Society for Parenteral and Enteral Nutrition: characteristics recommended for the identification and documentation of adult malnutrition (undernutrition). *J Acad Nutr Diet*. 2012;112(5):730-738.
191. Malone A, Hamilton C. The Academy of Nutrition and Dietetics/ the American Society for Parenteral and Enteral Nutrition consensus malnutrition characteristics: application in practice. *Nutr Clin Pract*. 2013;28(6):639-650.
192. Shizgal HM. Nutritional assessment with body composition measurements by multiple isotope dilution. *J Parent Enter Nutr*. 1990;17(3-suppl):9-17.
193. Wabel P, Chamney P, Moissl U, Jirka T. Importance of whole-body bioimpedance spectroscopy for the management of fluid balance. *Blood Purif*. 2009;27(1):75-80.
194. Cecconi M, Rhodes A, Poloniecki J, Della Rocca G, Grounds RM. Bench-to-bedside review: the importance of the precision of the reference technique in method comparison studies--with specific reference to the measurement of cardiac output. *Crit Care*. 2009;13:201.
195. Stover JF, Stocker R, Lenherr R, et al. Noninvasive cardiac output and blood pressure monitoring cannot replace an invasive monitoring system in critically ill patients. *BMC Anesthesiol*. 2009;9:6.
196. Van Der Kleij SCJ, Koolen BB, Newhall D a., et al. Clinical evaluation of a new tracheal impedance cardiography method. *Anaesthesia*. 2012;67(7):729-733.
197. Ward LC. Bioelectrical impedance validation studies: alternative approaches to their interpretation. *Eur J Clin Nutr*. 2013;67(S1):S10-S13.
198. Tengvall M, Ellegård L, Malmros V, Bosaeus N, Lissner L, Bosaeus I. Body composition in the elderly: reference values and bioelectrical impedance spectroscopy to predict total body skeletal muscle mass. *Clin Nutr*. 2009;28(1):52-58.
199. Ellis KJ, Wong WW. Human hydrometry: comparison of multifrequency bioelectrical impedance with 2H2O and bromine dilution. *J Appl Physiol*. 1998;85(3):1056-1062.
200. Vine SM, Painter PL, Kuskowski MA, Earthman CP. Bioimpedance spectroscopy for the estimation of fat-free mass in end-stage renal disease. *E Spen Eur E J Clin Nutr Metab*. 2011;6(1):e1-e6.